SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDY OF 1, 8-DIOXO-OCTAHYDROXANTHENE DERIVATIVES IN DIFFERENT AMBERLITE CATALYSTS

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Synthesis, Characterization and Antimicrobial Study of 1, 8-Dioxo-octahydroxanthene Derivatives in Different Amberlite Catalysts

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As thesis research advisors, we hereby certify that we have read and evaluated this thesis prepared, under our guidance, by Wubetu Belay entitled: "**SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDY OF 1, 8-DIOXO-OCTAHYDROXANTHENE DERIVATIVES IN DIFFERENT AMBERLITE CATALY-STS"**. We recommend that it be submitted as fulfilling the thesis requirement.

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As members of the board of examiners of the MSc thesis open defense examination, we certify that we have read and evaluated the thesis prepared by Wubetu Belay and examined the candidate. We recommended that the thesis be accepted as fulfilling the thesis requirement for the degree of Master of Science in Chemistry.

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DEDICATION

This thesis manuscript is dedicated to my beloved family for their inspiration, love and moral support throughout my life.

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ACRONYMS AND ABBREVIATIONS

AMC	Active methylene compound
^{13}C NMR	Carbon Nuclear Magnetic Resonance
DCC	DicyclohexylCarbodiimide
DEPT	Distortion Enhancement by Polarization Transfer
DMSO	Dimethyl sulphoxide
DNA	Deoxyribo Nucleic Acid
FTIR	Fourier Transform Infrared Spectroscopy
^{1} H NMR	Hydrogen Nuclear Magnetic Resonance
IER	Ion Exchange Resin
MHA	Mueller Hinton Agar
MDR	Multi-Drug Resistant
NNRTI	Non Nucleotide Reverse Transcriptase Inhibitors
NSAID	Non -Steroidal Anti-Inflammatory Drug
PDA	Potato Dextrose Agar
RNA	Ribo Nucleic Acid
RND	Resistance-Nodulation-Cell Division
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
UV	Ultraviolet

BIOGRAPHY OF THE AUTHOR

The author was born on September 5, 1991 in Amhara Region, West Gojam Zone in Yilmana Denssa Woreda. He first started his education at Sekela Primary School from 1997/8-2003/4 in Sekela. He then attended his secondary educations at Adet Senior Secondary and Preparatory School from 2004/5-2007/8. After successful completion of the preparatory education, he joined Hramaya University in 2008/9 and graduated with B.Sc. in Chemistry in 2010/11. After graduation, he was employed as a graduate assistant at Hramaya University under ULMD and then he joined the Postgraduate Program Directorate of Haramaya University in September, 2014/15 to pursue his MSc study in Chemistry (Organic Chemistry).

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ABSTRACT

The present work was carried out to synthesize the title compounds by using two different Amberlite catalysts viz. Amberlite IRA 67 and Amberlite IRC 86. The title compounds were synthesized in good yields under both catalysts. The structures of the as-synthesized compound were identified using IR and one dimensional NMR techniques such as ^{1}H NMR, ^{13}C NMR and DEPT-135. Comparison between the two catalysts with respect to percentage yield, reaction time, facile work up procedures and catalyst recovery evidenced Amberlite IRA 67 as better catalyst than Amberlite IRC 86. The synthesized compounds were tested against four bacterial species (two gram negative and two gram positive bacteria) and two fungal species (Fusarium oxysporum and Aspergillus niger) using paper disc diffusion method. Based on the findings of the present work out of the four bacteria species considered in this study, relatively good inhibition zone was evidenced for the three of them while Streptococcus agalactia doesn't show any inhibition zone. On the other hand, of the two fungal species Fusarium oxysporum was inhibited by the as-synthesized compound where as Aspergillus niger doesn't show any inhibition zone.

Key words: 1,8-Dioxooctahydroxanthene, aryl benzaldehyde, active methylene compound, Amberlite IRA 67, Amberlite IRC

1. INTRODUCTION

The process of establishing a new drug is exceedingly complex and involves talents of people from variety of disciplines. An important aspect of medicinal chemistry has been to establish a relationship between chemical structure and biological activity. Although many natural products are used in pharmaceuticals in their original chemical structures, successful efforts have been made to improve their pharmaceutic and therapeutic properties through structural modification so as to synthesize new molecules (Lakshika and Vinay, 2011).

For more than a century, heterocyclic compounds have been recognized as the most important organic compounds. They have contributed to the development of society from a biological and industrial point of view as well as to the understanding of life processes and to the efforts to improve the quality of life. They participate in most important biochemical processes and are the constituents of DNA and RNA in the living cells. It has been established that half of the therapeutic agents consist of heterocyclic compounds. The heterocyclic ring comprises the core of the active moiety or pharmacophore (Ghannoun, 2001).

Heterocyclic compounds are of massive importance in the design and discovery of new compounds for pharmaceutical applications (Kishi, 1993). The majority of pharmaceuticals and biologically active compounds contain heterocyclic moieties while countless additives and modifiers used in variety of applications. One of the striking structural features inherent to heterocycles, which was continued to be exploited to a great advantage by the drug industry, lies in their ability to manifest substituent around a core scaffold in well defined three-dimensional representations. Based upon these considerations a family of new compounds known as dendrils have come into existence having wide range of applications *(*Niknam and Damya, 2009).

Heterocyclic compounds are cyclic compounds in which one or more of the atoms of the carbon ring are heteroatoms (atoms other than carbon). The name comes from the Greek word heteros, which means “different.” A variety of atoms, such as N, O, S, Se, P, Si, B, and As, can be incorporated into ring structures (Lakshika and Vinay, 2011).

Oxygen heterocycles is the second major class of organic heterocyclic compounds. Many naturally occurring oxygen heterocycles such as sugars, vitamins, hormones, antibiotics and

pigments are biologically active compounds (Parminderjit *et al.,* 2013).The oxygen containing heterocycles are classified on the basis of the structure of ring and position of oxygen in the ring such as Phenoxazine, Xanthene, Benzofuran, chromene, Pyrilium, Coumarine *(*Niknam and Damya, 2009).

Xanthenes have received significant attention by many pharmaceutical and organic chemists essentially because of the broad spectrum of their biological and pharmaceutical properties such as antiviral, antibacterial (Safaei- Ghomi and Ghasemzadeh, 2015), anti-inflammatory activities (Poupelin,1978), as well as efficiency in photodynamic therapy (Ion, 1997) and antagonist for the paralyzing action of zoxazolamine (Kumar *et al.,* 2006). Xanthenes are also an important class of organic compounds that finds use as leuco dyes *(*Niknam and Damya, 2009). PH-sensitive fluorescent materials for visualization of biomolecules (Hunter and Beveridge, 2005) and in laser technologies (Ahmad *et al.,* 2002*)* due to their useful spectroscopic properties.

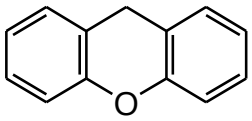


Figure 1. General structure of xanthene

1, 8-Dioxo-octahydroxanthenes is one of xanthenes derivative that have been found in a large number of naturally occurring molecules. They have received great attention because of their therapeutic (Wang *et al.,* 1997) and biological properties such as, antiviral (Poupelin, 1978), anti inflammatory (Safaei- Ghomi and Ghasemzadeh, 2015), antibacterial (Ion, 1997), antifungal (Ihsan Ali, 2014), photodynamic therapy (Kumar *et al.,* 2006). Xanthenes are pH sensitive fluorescent materials and due to this property they are used for the visualization of biomolecular assemblies (Knight and Stephens, 1989).

There are several reports in the literature for the synthesis of 1,8-dioxooctahydroxanthene derivatives employing aromatic aldehydes and 5,5-dimethyl-1,3-cyclohexanedione, these includes; $InCl_3.4H_2O$ in ionic liquid (Fan *et al.,* 2005), solid state condensation by grinding at room temperature (Dharma Rao *et al.,* 2012), diammunium hydrogen phosphate (Darviche *et al.,* 2007), p-dodecylbenzenesulfonic acid in water (Jin, *et al.,* 2004), Fe^{3+}-montmorillonite (Song *et al.,* 2007), $NaHSO_4$-SiO_2 or silica chloride, amberlyst-15 (Das *et al.,* 2007), silica sulfuric acid,

(Seyyedhamzeh *et al.,* 2008), tetrabutylammonium hydrogen sulfate, trimethylsilylchloride (TMSCl) (Karade *et al.,* 2007), montmorillonite K-10-supported (Sharifi *et al.,* 2008) and 1-butyl-3-methylimidazolium hydrogen sulfate (Rashedian *et al.,*2010). Each of these methods have their own advantages but also some of them often suffer from one or more disadvantages such as prolonged reaction time, tedious work-up processes, low yield, lack of easy availability/preparation of starting materials, expensive reagents and hazardous reaction conditions. Hence, a more realistic catalyzed condensation between active methylene carbonyl compound and aldehyde is needed for contemporary chemical synthesis with less waste and more facile isolation of products, perhaps with reuse of the catalysts as well.

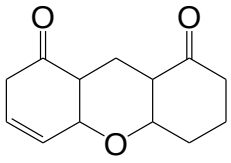


Figure 2. General structure of 1, 8-dioxo-octahydroxanthene

It is important to find more efficient catalysts for the synthesis of these types of compounds. Although 1, 8-dioxo-octahydroxanthene owing to their high versatility in exhibiting diverse potent biological, dyeing and ligation properties have been synthesized and characterized consistently for several decades, diversity in their uses infuses considerable interest in their chemistry. With a view to further assess the microbial profile of this class of compounds, it has been thought worthwhile to synthesize, characterize and screen for some antimicrobial (antibacterial and antifungal) properties of some new compounds, obtained by cyclocondsetion of Schiff's bases derived from arylaldehyde and dimedone. In the present study, some 1, 8-dioxo-octahydroxanthene derivatives were synthesized using Amberlite IRA 67 and amberlite IRC 86 as catalysts to find out best set of conditions and to get a good yield of the synthesized compound.

General Objectives

- Synthesis of 1, 8-Dioxo-octahydroxanthenes using different amberlite catalysts, their characterization and antimicrobial screening

Specific Objectives

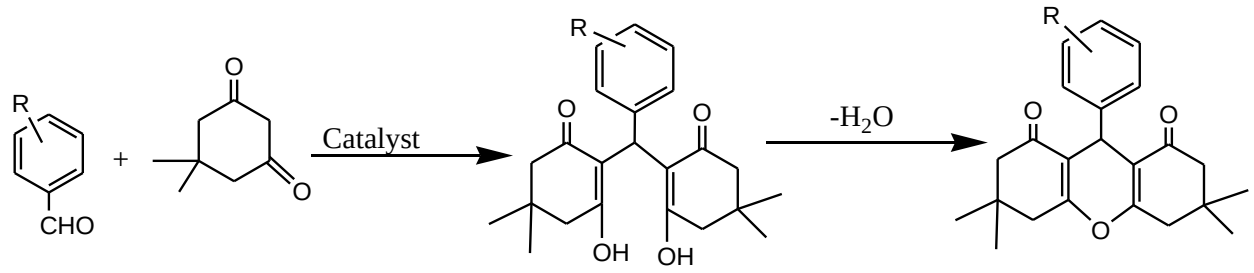
- To synthesize 1, 8-Dioxo-octahydroxanthenes using Amberlite IRA 67 and Amberlite IRC 86 as a catalyst
- To find out "best set of conditions" for synthesis of 1, 8-Dioxo-octahydroxanthene with respect to catalysts
- To characterize the structure of synthesized 1, 8-Dioxo-octahydroxanthenes by using ^{13}C, ^{1}H, DEPT-135 and IR spectroscopic techniques
- To investigate antimicrobial activities of 1, 8-Dioxo-octahydroxanthenes using the disc diffusion method on selected bacterial and fungal species

2. REVIEW OF LITERATURE

2.1. Heterocyclic Compounds

Heterocyclic compounds are cyclic compounds in which one or more of the atoms of the carbon ring are heteroatoms. A heteroatom is an atom other than carbon. The name comes from the Greek word heteros, which means "different." A variety of atoms, such as N, O, S, Se, P, Si, B, and As, can be incorporated into ring structures. By far the most numerous and most important heterocyclic systems are those having five and six members. More than half of all known organic compounds are heterocyclic. Almost all the compounds we know as drugs, vitamins, and many other natural products are heterocyclic (Lakshika and Vinay, 2011).

Xanthenes have received significant attention from many pharmaceutical and organic chemists essentially because of the broad spectrum of their biological and pharmaceutical properties such as antiviral, antibacterial, (Safaei- Ghomi and Ghasemzadeh, 2015) anti-inflammatory activities, as well as efficiency in photodynamic therapy (Pravin, 2011) and antagonist for the paralyzing action of zoxazolamine (Mohammad Reza *et al.,* 2013). There are several reports in the literature for the synthesis of 1,8-dioxooctahydroxanthene derivatives employing aromatic aldehydes and 5,5-dimethyl-1,3- cyclohexanedione, these include $InCl_3$. $4H_2O$ in ionic liquid, $FeCl_3.6H_2O$ in ionic liquid, (Fan *et al.*, 2005) solid-state condensation by grinding at room temperature, NH_2SO_3H in aqueous media (Dharma Rao *et al.*, 2012), microwave-induced synthesis in solid or liquid phase, p-dodecylbenzenesulfonic acid in water (Tu *et al.*, 2001). Each of these methods have their own advantages but also suffer from one or more disadvantages such as prolonged reaction time, tedious work-up processes, low yield, lack of easy availability/preparation of starting materials and hazardous reaction conditions. In addition, chemo selectivity can be a problem, if acid sensitive groups are present in the same molecule. The major disadvantage of some of the methods is that the reaction does not go to completion and stops at open chain structure [2,2′-arylmethylene bis(3-hydroxy-5,5-dimethyl-2- cyclohexene-1-one)], (Hitendra *et al.,* 2007), instead of forming the cyclized compound.

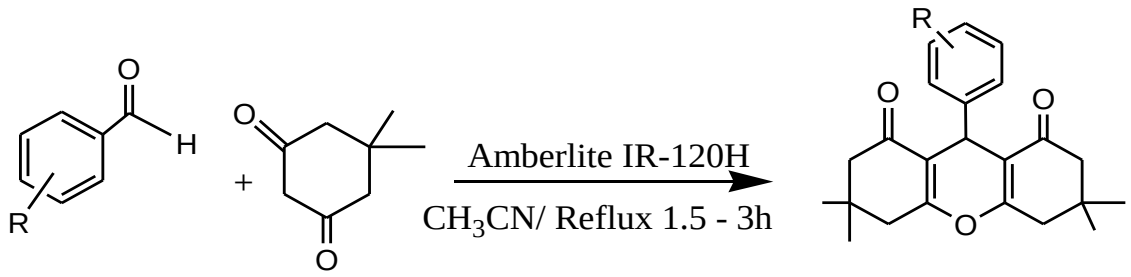


Where R= H, 4-Cl, 4-NO_2, 3-NO_2, 4-OH, 4-OMe, 2-Cl ...

Figure 3. General reaction for the Synthesis of xanthenedione derivatives

2.2. Synthesis of 1, 8-dioxo-octahydroxanthene

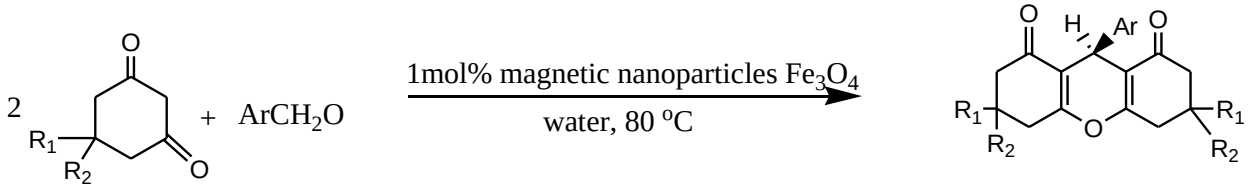
The main synthetic route to prepare 1,8-dioxo octahydroxanthenes involve three components which are aldehyde, active methylene group and a catalyst with a certain step process. In recent years, several methods are reported for the preparation of 1,8-dioxo octahydroxanthenes such as cyclocondensation between 2-hydroxy aromatic aldehydes and 2-tetralone (Knight and Little, 2001) trapping of benzenes by phenols, cyclization of polycyclic aryltriflate esters (Kuo and Fang, 2001), and intramolecular phenyl carbonyl coupling reactions of benzaldehyds and acetophenones (Jha and Beal, 2004). The conventional procedure involves acid- or base-catalyzed condensation of appropriate active methylene carbonyl compounds like dimedone with aldehydes (Hangirgekar *et al.*, 2014), in the presence of p-dodecylbenzenesulphonic acid (Jin *et al.,* 2004), Fe^{3+}-montmorilonite (Song *et al.,* 2007), $NaHSO_4$-SiO_2 or silica chloride (Shankar *et al.,* 2014), Amberlyst-15 (Balaji *et al.,* 2010), $(NH_4)_2HPO_4$ (Darviche *et al.,* 2007), Dowex-50W (Shakibaei *et al.,* 2007), $SbCl_3/SiO_2$ (Zhang and Liu, 2009), and $BiCl_3$ (Li *et al.,* 2008), as well as with the assistance of ultrasound (Venkatesan *et al.,* 2008) or microwave irradiation (Hua *et al.,* 2005) have been reported.



Where R = *3*-NO_2,*4*-F, *4*-Cl, *4*-OH, *4*-NO_2, *4*-$N(CH_3)_2$, *4*-OCH_3, *4*-OH, *3*-OCH_3, *4*-H

Figure 4. Amberlite IR-120H catalyzed synthesis of 1,8-dioxo-octahydroxanthene derivatives

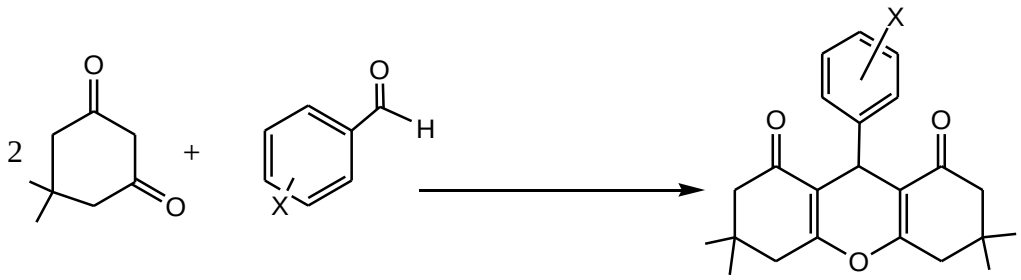
One method for the synthesis of 1,8-dioxo-octahydroxanthenes was obtained by condensation of 1,3-cyclohexanediones and benzaldehydes in the presence of magnetic nanoparticles Fe_3O_4 as an effective catalyst in water as a solvent. A mixture of aromatic aldehydes, 1,3-cyclohexanediones and magnetic nanoparticles Fe_3O_4 in H_2O was stirred at 80 ^{0}C in the oil bath (Darwishand Wills, 2012).



R_1 = H, Me
R_2 = H, Me

Figure 5. Synthesis of 1,8-dioxo-octahydroxanthenes in the presence of 1,3-cyclohexanediones, benzaldehydes and magnetic nanoparticles Fe_3O_4

The synthesis of 1,8-dioxooctahydroxanthene have been reported also catalyzed by cellulose sulfonic acid as a heterogeneous and reusable ecofriendly catalyst under solvent-free conditions.



where X = H, 4-OH, 4-Br, 3-NO_2, 3-NO_2, 4-Cl, 4-OCH_3, 4-CH_3

Figure 6. Synthesis of 1,8-dioxooctahydroxanthene by using cellulose sulfonic acid as a catalyst

Mulakayala and his co-workers 2012, have been reported also synthesis of 1,8-dioxooctahydroxanthene catalyzed by ceric ammonium nitrateas catalyst under Ultrasound.

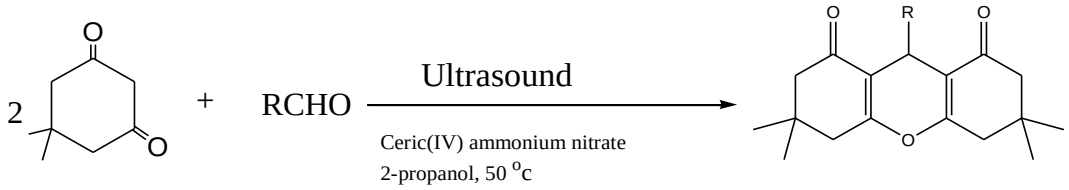


Figure 7. Ultrasound assisted one pot synthesis of 1,8-dioxo-octahydroxanthene derivatives using ceric ammonium nitrate as a catalyst

2.3. CATALYSTS

Catalysis is a phenomenon by which chemical reactions are accelerated by small quantities of foreign substances, called catalysts. A suitable catalyst can enhance the rate of a thermodynamically feasible reaction but cannot change the position of the thermodynamic equilibrium. Most catalysts are solids or liquids, but they may also be gases. The catalytic reaction is a cyclic process. According to a simplified model, the reactant or reactants form a

complex with the catalyst, thereby opening a pathway for their transformation into the product or products. Afterwards the catalyst is released and the next cycle can proceed.

2.3.1. Types of Catalysis

2.3.1.1. Homogeneous Catalysis

In homogeneous catalysis, both the catalyst and the reactants are in the same phase, i.e. all are molecules in the gas phase, or, more commonly, in the liquid phase. One of the simplest examples is found in atmospheric chemistry. Ozone in the atmosphere decomposes, among other routes, via a reaction with chlorine atoms: Metal salts of organic acids, organometallic complexes, and carbonyls of Co, Fe, and Rh are typical homogeneous catalysts.

2.3.1.2. Heterogeneous Catalysis

Heterogeneous catalysis involves systems in which catalyst and reactants form separate physical phases. Heterogeneous catalysts are present in a different phase, usually solid. The main advantage of using a heterogeneous catalyst is the relative ease of catalyst separation from the product stream that aids in the creation of continuous chemical processes. Additionally, heterogeneous catalysts are typically more tolerant of extreme operating conditions than their homogeneous analogues. Typical heterogeneous catalysts are inorganic solids such as metals, oxides, sulfides, and metal salts, they may also be organic materials such as organic hydro peroxides, ion exchangers, and enzymes.

IER are types of heterogeneous catalysts and are insoluble polymers that contain acidic or basic functional groups and have the ability to exchange counter-ions within aqueous solutions surrounding them. An ion exchange resin is exhibited like small bead with a diameter between 1-2 mm. It is usually white or yellowish and it is fabricated from an organic polymer substrate backbone. Ion exchange is a reversible process in which ions of like sign are exchanged between liquid and solid when in contact with a highly insoluble body. Due to the presence of high molecular weight water insoluble polymers, the resins are not absorbed by the body and are therefore inert. IER have specific properties like available capacity, acid base strength, particle size, porosity.

A major advantage of ion exchange system is low running cost. It requires little energy and the regenerated chemicals are cheap. Furthermore, if well maintained, resin beds can last for many years before replacement. However, the limitation is that the release rate is proportional to the concentration of the ions present in the area of administration.

Structure and Chemistry of Ion Exchange Resin: are simply insoluble polyelectrolytes that are insoluble polymers which contain ionizable groups distributed regularly along the polymer backbone. The most common resins used in formulations are cross-linked polystyrene and polymethacrylate polymers. When IER are mixed with a fluid such as water, ions in the fluid can exchange with the polyelectrolyte's counter ions and be physically removed from the fluid.

An ion exchange resin is a polymer (normally styrene) with electrically charged sites at which one ion may replace another. There are numerous functional groups that have charge, only a few are commonly used for man-made IER. These are:

-COOH, which is weakly ionized to $-COO^{-}$,

$-SO_3H$, which is strongly ionized to $-SO_3^{-}$,

$-NH_2$, which weakly attracts protons to form NH_3^{+},

-secondary and tertiary amines that also attract protons weakly,

$-NR_3^{+}$, which has a strong, permanent charge (R stands for some organic group).

These groups are sufficient to allow selection of a resin with either weak or strong positive or negative charge.

Types of Ion-exchange Resins: There are two major classes of ion-exchange polymers. These are Cation exchange resin and anion exchange resin.

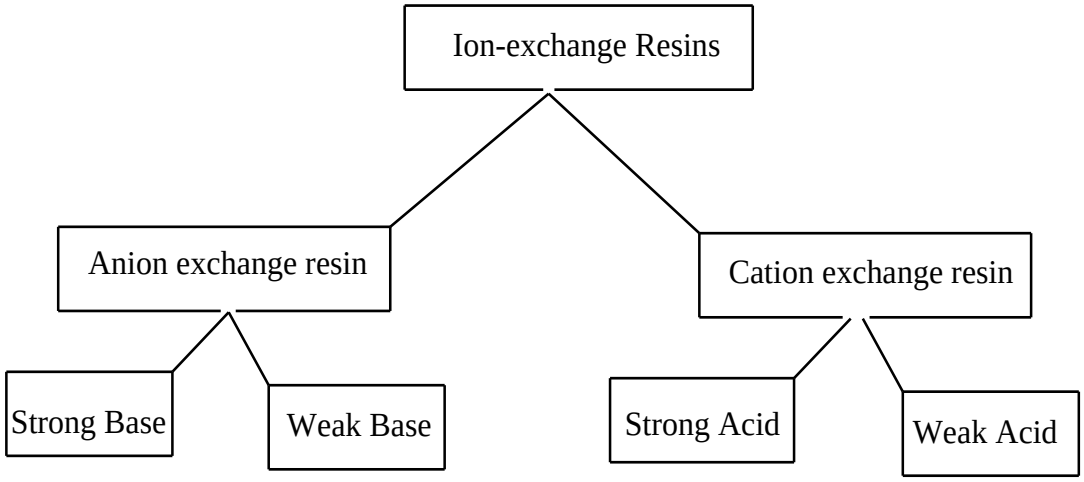


Figure 8. Classification of ion-exchange resins

Cation exchange resins: Cation exchange resins contain covalently bound negatively charged functional groups and exchanges positively charged ions. There are prepared by the copolymerization of styrene and divinyl benzene and have sulfonic acid groups ($-SO_3H$) introduced into most of the benzene rings). The mechanism of cation exchange process can be represented by the following reaction

$$R^- - ex^+ + C^+ \rightarrow R^- - C^+ + ex^+$$

where, R is a resin polymer with SO_3^- sites available for bonding with exchangeable cation (ex^+), and C^+ represents a cation in the surrounding solution getting exchanged

A typical cation exchange resin is prepared by the copolymerization of styrene and divinylbenze. During the polymerization, polystyrene formed as a linear chains and these become covalently bonded to each other by divinylbenze cross links. If sulphuric acid is then allowed to react with this copolymer, sulphonic acid groups are introduced into most of the benzene rings of the styrene-divinylbenze polymer, and the final substance formed is known as cation-exchange resin (Srikanth, M. V. et al., 2010).

I II

Figure 9. Chemical structure of (I) styrene (II) divinyl benzene.

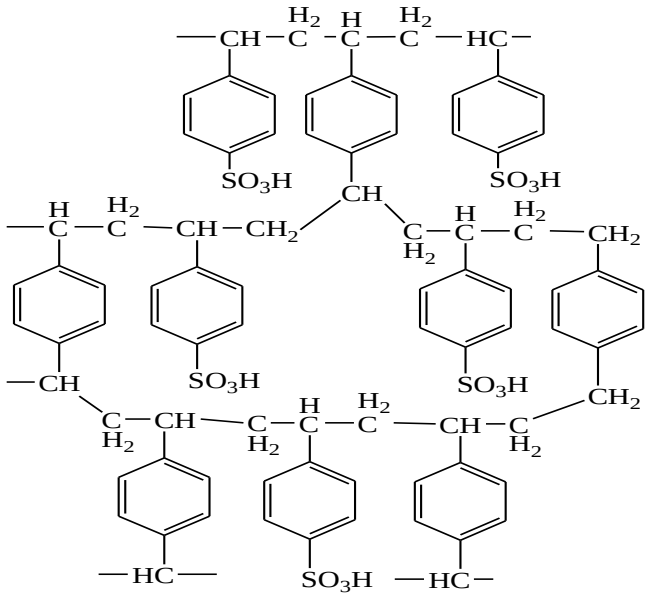


Figure 10. Chemical structure of a cation exchange resin

Cation exchange resins can be further classified into strong acid cation exchange resins and weak acid cation exchange resins.

Strong acid cation exchange resins: have the chemical behavior similar to that of a strong acid. These resins are highly ionized in both the acid ($R\text{-}SO_3H$) and salt (RSO_3Na) form of the sulfonic acid group ($\text{-}SO_3H$). They can convert a metal salt to the corresponding acid by the reaction in Eq. below:

$$2(R\text{-}SO_3H) + NiCl_2 \rightarrow (R\text{-}SO_4)\ Ni + 2HCl$$

The hydrogen and sodium forms of strong acid resins are highly dissociated, and the exchangeable Na^+ and H^+ are readily available for exchange over the entire pH range. Consequently, the exchange capacity of strong acid resins is independent of the solution pH. DIAION^{tm} SK, PK, and HPK series belong to the classifications of Strong acid cation exchange resins.

Weak acid cation exchange resins: behave similarly to weak organic acids that are weakly dissociated. In a weak acid resin the ionisable group is a carboxylic acid (COOH) as opposed to the sulfonic acid group (SO_3H) used in strong acid resins. The degree of dissociation of a weak acid resin is strongly influenced by the solution pH. Consequently, resin capacity depends in part on the solution pH. A typical weak acid resin has limited capacity below a pH of 6.0, making it unsuitable for deionizing acidic metal finishing wastewater.

Amberlite IRC86 resin is a gel type high capacity weak acid cation exchange resin containing carboxylic acid groups. Karthika C. and Sekar M., 2012; investigated that, Amberlite IRC-86 used to remove Hg(II) ions from aqueous solutions. Swelam *et al*., 2015; have reported that Amberlite IRC 86 Ion-Exchange Resins are used for removing target pollutant Ni(II) from aqueous solutions and focus on the binding equilibrium and batch studies of metal ions.

Table 1. Properties of Amberlite IRC 86 cation exchange resin

Physical form	Clear amber spherical beads
Matrix	Gel polyacrylic copolymer
Functional group	Carboxylic acid
Ionic form as shipped	H^+
Total exchange capacity	≥ 4.10 eq/L (H^+ form)
Moisture holding capacity	47 to 53 % (H^+ form)
Shipping weight	790 g/L
Particle size	
Uniformity coefficient	≤ 1.80
Harmonic mean size	0.580 to 0.780 mm < 0.300 mm 2.0 % max
Reversible swelling (total conversion)	$H^+ \rightarrow Na^+ \leq 100$ % $H^+ \rightarrow Ca^{++} \leq 15$ % $H^+ \rightarrow Mg^{++} \leq 50$ %

Anion exchange resins: Anion exchange resins have positively charged functional groups and there exchanges negatively charged ions. These are prepared by first chlormethylating the benzene rings of styrene-divinylbenzene copolymer to attach CH_2Cl groups and then causing these to react with tertiary amines such as triethylamine. The mechanism of anion exchange process can be represented by the following reaction in Eq. below.

$R^+ - ex^- + A^- \rightarrow R^+ - A^- + ex^-$

where, R^+ indicates a resin polymer with number of sites available for bonding with exchangeable anion (ex^-), and A^- indicates cations in the surrounding solution getting exchanged.

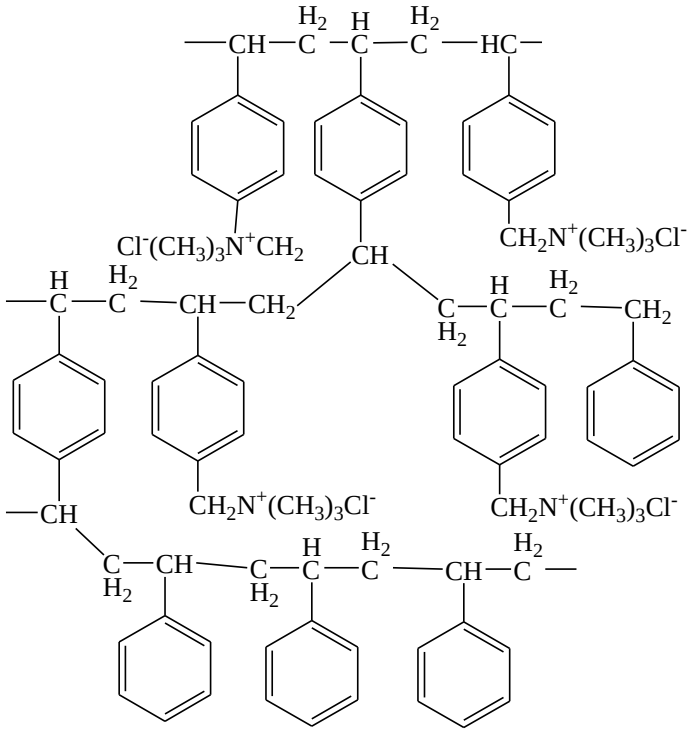


Figure 11. Chemical structure of an Anion exchange resin

Anion exchange resins can be further classified into two which are:

Strong base anion exchange resins: are highly ionized and can be used over the entire pH range. These resins are used in the hydroxide (OH) form for water deionization. They will react with anions in solution and can convert an acid solution to pure water.

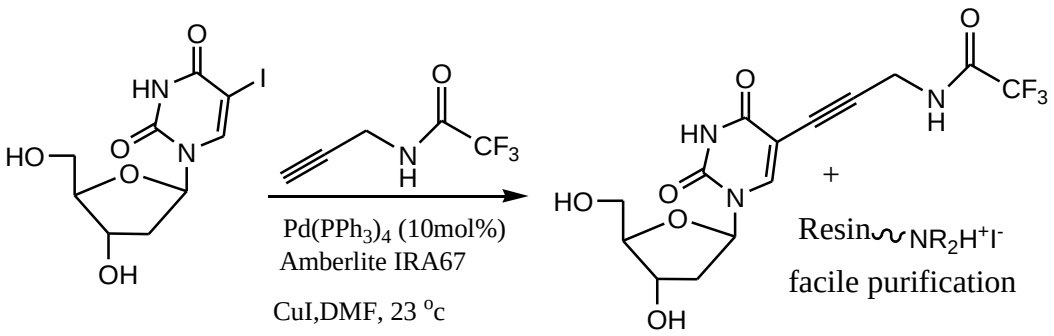
$R\text{-}NH_3OH + HCl \rightarrow R\text{-}N^+H_3Cl + H_2O$

Regeneration with concentrated sodium hydroxide (NaOH) converts the exhausted resin to the OH form. IRA 400 &IRA 900 are some examples of Strong base anion exchange resins.

Weak base anion exchange resin: are like weak acid resins in that the degree of ionization is strongly influenced by pH. Hence, weak base resins exhibit minimum exchange capacity above a pH of 7.0. The weak base resin does not have an OH ion form as does the strong base resin.

$R\text{-}NH_2 + HCl \rightarrow R\text{-}NH_3Cl$

Amberlite IRA 67 resin is a weak base anion exchange resin with a gel type acrylic matrix. It has a high capacity, excellent physical stability, fast kinetics, outstanding resistance to organic fouling and basicity higher than that of polystyrenic weak base resins. Firth *et al.,* 2011; reported from Interrogation of a Sonogashira cross-coupling of 8-bromoguanosine with phenylacetylene on Amberlite: evidence for Pd/Cu Ion binding and propagation of Pd/Cu nanoparticles that, there are several advantages associated with the use of Amberlite IRA 67 in Sonogashira cross couplings. The Amberlite IRA 67, possessing dimethylamino groups on the resin surface, will sequester Pd and Cu species, also effectively competing with guanosine coordination, facilitates removal of these metal residues by filtration on reaction completion. The use of Amberlite, reduces the Pd, Cu, and iodide content in the final product. Hence, the use of the solid-supported base on Amberlite IRA 67 improves the separation of both the metals and residual halide.



(79% yield)

Figure 12. The polar nucleoside product from triethylammonium salts formed from the Sonogashira coupling of iodide with protected propargylamine by using Amberlite IRA 67 as a catalyst.

Table 2. Properties of Amberlite IRA 67 anion exchange resin

Physical form	Translucent white spherical beads
Matrix	Crosslinked acrylic gel structure
Functional group	Tertiary amine
Ionic form as shipped	Free Base (FB)
Total exchange capacity	≥ 1.60 eq/L (FB form)
Moisture holding capacity	56 to 64 % (FB form)
Shipping weight	700 g/L
Particle Size	
Uniformity coefficient	≤ 1.80
Harmonic mean size	0.500 – 0.750 mm < 0.300 mm 3.0 % max
Reversible swelling	FB → Cl^- ≤ 30 %

2.4. Characterization Methods

2.4.1. NMR Spectroscopic Methods

Proton NMR is a plot of signals arising from absorption of radio frequency during NMR experiment by the different protons in a compound under study as a function of frequency (chemical shift). The area under the plots provides information about the number of protons present in the molecule, the position of the signals reveals information regarding the

chemical and electronic environment of the protons, and the splitting pattern provides information about the number of neighboring (vicinal or germinal) protons (Kalsi, 2004).

Carbon – 13 NMR is similar to proton NMR. It is a plot of signals arising from the different carbons as a function of chemical shift. The signals in ^{13}C-NMR experiments normally appear as singlet's because of the decoupling of the attached protons. The ^{13}C spectrum provides important structural information. It also confirms the presence of carbonyl groups and other non protonated carbon atoms whose presence in a molecule can only be inferred from the 1H-NMR spectrum (Kalsi, 2004).

The range of chemical shift values differs between 1H (normally 0 - 10) and ^{13}C NMR (normally 0 - 230) that arises from the two nuclei having different numbers of electrons around corresponding nuclei and different electronic configurations (Sanders and Hunter, 1993).

DEPT-135 Currently, the most commonly used method for determining the number of hydrogen bonded carbon atom is the DEPT experiment. The DEPT-135 spectrum again shows all protonated carbon signals with CH_3 and CH resonances being positive, whereas CH_2 signals are negative. The quaternary carbons do not give signals in DEPT-135.

2.4.2. IR Spectroscopic Method

The term "infrared" generally refers to any electro-magnetic radiation falling in the region from 0.7 μm to 1000 μm. However, the region between 2.5 μm and 25 μm (4000 to 400 cm^{-1}) is the most attractive for chemical analysis. This "mid-IR" region includes the frequencies corresponding to the fundamental vibrations of virtually all of the functional groups of organic molecules. These spectral lines are typically narrow and distinct, making it possible to identify and monitor a band corresponding to the specific structural feature that is to be modified by a reaction. As a result, quantitative calibrations performed in the mid-IR are usually straightforward and robust, being largely immune to the effects of spurious artifacts.

2.5. Biological Activities of Xanthene and Related Compounds

The main objectives of organic and medicinal chemistry are to design and synthesize molecules having value as human therapeutic agents. During the past decade, combinatorial chemistry has provided access to chemical libraries based on privileged structures (Horton *et al.,* 2003), with heterocyclic ring which are receiving special attention as they belong to a class of compounds with proven utility in medicinal chemistry (Thompson and Ellman, 1996). It is evident from literature that isatin derivatives are known to be associated with broad spectrum of biological activities like anti-hiv, anti-cancer, anti-microbial, anticonvulsant, and anti-inflammatory activities. Following examples of biological properties of octahydroxanthenederivatives manifest their medicinal importance.

2.5.1. Antimicrobial Activity

2.5.1.1. Antibacterial Activity

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cohen, 1992). Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient (Gislene *et al.,* 2000).Pathogenic bacteria have always been considered as a major cause of morbidity and mortality in humans. Even though pharmaceutical companies have produced a number of new antibacterials in the last years, resistance to these drugs has increased and has now became a global concern. The global emergence of multi-drug resistant (MDR) bacteria is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure. Bacterial resistance to chemically unrelated antimicrobial agents is public health concern

and may be caused by over-expression of MDR efflux pumps. In Gramnegative bacteria, the effect of the efflux pumps in combination with the reduced drug uptake (due to the presence of a double membrane barrier) is responsible for the high inherent and acquired antibiotic resistance often associated with this group of organisms. Among Gramnegative bacteria, many of these MDR efflux pumps belong to the RND (resistance-nodulation-cell division) type family of tripartite efflux pumps (Djeussi *et al.,* 2013). Kale and Burungale, 2012; have synthesized 3,3,6,6-tetramethyl-9-(3-nitro-phenyl)-3,4,5,6,7,9-hexahydro-2H-xanthene-1,8-dione and tested against specific bacterial strains like: sa*lmonella abony, Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In case of *Salmonella abony* the compound is active in sterile water while *Escherichia coli* show positive activity in DMSO solvent.

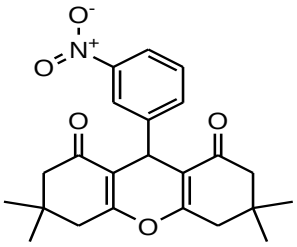


Figure 13. 3,3,6,6-tetramethyl-9-(3-nitro-phenyl)-3,4,5,6,7,9-hexahydro-2H-xanthene-1,8-dione

2.5.1.2. Anticancer Activity

Most of the xanthene derivatives synthesized for tested for their anticancer properties in vitro and for their anti-proliferative properties in vitro against a number of cancer cell lines for example, human chronic myeloid leukemia cells, human colon carcinoma, and human neuroblastoma cells. 1,8-dioxo-octahydroxanthenes have medicinal value and the basic framework of this class of heterocycles could be an attractive template for the identification of novel and potential anticancer agents (Mulakayala *et al.*, 2012).

Among the myriad of biological activities described for xanthones, the *in vitro* growth inhibitory activity on tumor cell lines appeared to be quite remarkable, since they are biologically active on a wide range of tumor cell lines (Pinto *et al.*,2005). Apart from the anti-tumor activity, some xanthones have been described for their anti-mutagenic effects (MacKeen *et al.*, 2000) and for

their cancer chemo-preventative effects, acting as inhibitors of tumor promotersinvitro(Ito *et al*., 2000) and in vivo (Saha *et al.*, 2004).

Cancer is a disease characterized by uncontrolled multiplication and spread of abnormal forms of the body's own cells. From literature survey it is well known that isatinheterocycles exhibit manifold importance in the field of medicinal chemistry as a potent chemotherapeutic agent. Wang have synthesized a series of 1, 8-Dioxo-Octahydroxanthene and evaluated their ability to inhibit prostate cancer cells and were detected, which were effective in killing prostate cancer cells (Wang *et al.*, 1997).

2.5.1.3. Antifungal Activity

Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest. In fruit and vegetables, there is a wide variety of fungal genera causing quality problems related to aspect, nutritional value, organoleptic characteristics, and limited shelf life (Dellavalle1 *et al.,* 2011). In addition, in some cases fungi are indirectly responsible for allergic or toxic disorders among consumers because of the production of mycotoxins or allergens. Generally, phytopathogenic fungi are controlled by synthetic fungicides; however, the use of these is increasingly restricted due to the harmful effects of pesticides on human health and the environment (Harris *et al.,* 2001). The increasing demand of production and regulations on the use of agrochemicals and the emergence of pathogens resistant to the products employed, justifies the search for novel active molecules and new control strategies. Fungi are ubiquitous in the environment, and infection due to fungal pathogens has become more common. The genus Alternaria Nees is widely distributed in nature and its species are among the most common fungi on the phyllosphere. It includes both plant-pathogenic and plant-saprophytic species that may damage crops in the field or cause post-harvest decay (Griffin and Chu, 1983), causing considerable economics losses for farmers and food industries. In addition, the genus produces mycotoxins and phytotoxins, and studies in the last decade have emphasized its toxicogenic properties rather than simply those that cause spoilage. The toxins alternariol, alternariol methyl ether, altenuene, and tenuazonic acid are known as possible food contaminants with potential toxicological risk (Pose *et al*., 2004). Xanthene itself is used as a fungicide in both agriculture and to fight fungal infections in animals (Ihsan, 2014).

2.5.1.4. Anti-inflammatory Activity

Inflammation is a normal, protective response to tissue injury caused by physical trauma, noxious chemicals or microbial agents. For anti-inflammatory activity; carrageenan induces for acute and formalin induces for chronic inflammation and these are applied to paw edema. A long term administration of anti-inflammatory drugs is necessary for chronic diseases such as osteoarthritis and rheumatoid arthritis (Poupelin *et al.*, 1978). The most widely used non-steroidal anti-inflammatory drugs (NSAID) or steroids suffer from several side effects. Hence, the search for effective anti inflammatory agents that could be safely used on a long term basis is a priority (John, 2002). Seyyedeh and Farhad, 2015; have reported that 1,8-Dioxo-octahydroxanthene derivatives are effective for Anti-inflammatory activity.

2.5.1.5. Antimalarial Activity

The great panacea of malaria treatment would be the development of a long lasting vaccine. Xanthones express potent activity against the malaria parasite *Plasmodium falciparum* and in particular chloroquine resistant strains (Riscoe *et al*., 2005), making them important leads in the discovery of the next anti-malarial drugs.

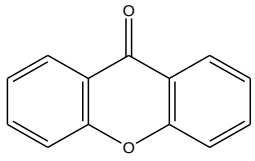


Figure 14. General Structure of Xanthone

Malaria is caused by protozoan parasites of the genus *Plasmodium*, which specifically attack erythrocytes. *Plasmodium falciparum* is responsible for over 80% of malaria cases worldwide and it causes the most severe, often fatal form of the disease (Riscoe *et al.*, 1997). The digestive vacuole is an acidic proteolytic compartment central to the metabolism of the *plasmodium* parasite and may be considered as its Achilles heel (Luzzi *et al.*, 1993). In this vacuole haemoglobin is degraded to provide amino acids for parasite growth in a process known as

hemoglobinolysis. Hemoglobinolysis also yields toxic heme , which serves as a reservoir of iron for ferroproteins. Although most of the heme is detoxified via polymerization into insoluble hemozoin (Riscoe *et al.*, 1997).Riscoe and co-workers have synthesized the xanthone 3,6-bis(ω-*N*,*N*diethylaminoamyloxy)- 4,5-difluoroxanthone was tested against multidrug resistant malaria strains of *Plasmodium falciparum.*

3. MATERIALS AND METHODS

3.1. Experimental Sites

Most of the experiments including synthesis and characterization of the new products were performed at Haramaya University, Central Laboratory as well as at Addis Ababa University. Furthermore, the investigation of the microorganisms was conducted at Pathology Laboratory in the School of Plant Science at Haramaya University.

3.2. Equipment and Chemicals

3.2.1. Equipment

The glassware and instruments which were used in this study are: measuring cylinder, beakers (different size), pipettes (different size), condenser, magnetic stirrers, wash brushes, burettes, glass rods, conical flasks (different size), round bottom flask (different size one necked and three necked), separator funnels, electronic balance, water bath, oven, TLC plates, rotatory evaporator, filter papers, melting point apparatus (Bibby Starling LTD , ST150SA model,U.K), thermometer and condensers.

3.2.2. Chemicals

The chemicals which were used in this study are: acetonitrile (99.9%, Park, UK), ethanol (99.9% Aldrich), dimedone, benzaldehyde, salicylaldehyde (99%, BDH, England), *p*-dimethylamino benzaldehyde (99%, Blulux, India), acetone (99%, Blulux, India), *n*-hexane (99%, Neolab), ethyl acetate (98%, Carol Erba), chloroform, dichloromethane (99.5%, Blulux, India), anhydrous $MgSO_4$, silica gel (70–230 mesh, E. Merck), Amberlite catalysts of (IRC 86 and IRA 67) for the synthesis of the target compound. Chloramphenicol, Bavistin, Potato dextrose agar (Micro master laboratories, India), Muller-Hinton agar (Aldrich chemical company, Germany), were used for the antimicrobial studies.

3.3. Physical and Spectroscopic Methods (Instrumentation)

Melting points of the synthesized compounds **(3a-3c)** were determined with electro-thermal melting point (England) without calibration. Column chromatography was performed using silica

gel (70–230 mesh, E. Merck) to afford the desired purity of the target molecules. IR spectroscopic analysis was done on a Shimadzu-IR-460 spectrometer using KBr pellets, and were recorded in the 500-4000 cm^{-1}. The ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra were recorded on a Bruker Avance Drx, operating at 600 MHz in $CDCl_3$ solvent, and the values are reported in terms of δ(ppm). TMS was used as the internal standard. Thin layer chromatography (TLC) was performed on pre-coated silica gel aluminium plates (Kieselgel 60, 254, E. Merck, Germany). The solvents used throughout this work were commercially available without purification. The other chemicals used for synthesis were obtained from Merck, TCI, Aldrich, Blulux and were used as received without purification.

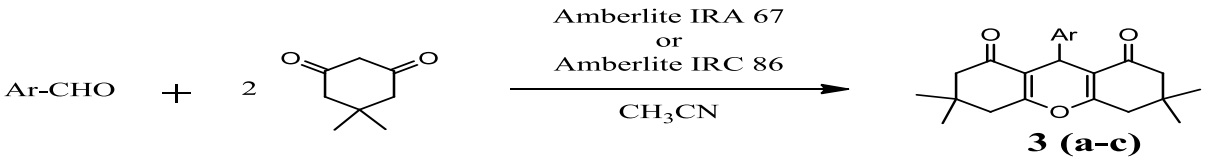
3.4. Determination of Melting Points

Melting points of synthesized compounds were determined in open capillaries tube using melting-point apparatus and are uncorrected. A few crystals of the synthesized 1,8-dioxooctahydroxanthene derivatives **(3a-3c)** were placed in a thin walled capillary tube 10-15 cm long, about 1 mm inside diameter, and closed at one end. The capillary was then inserted into melting-point apparatus to determine melting point of compounds.

3.5. General Procedure for the Synthesis of 1,8-Dioxooctahydroxanthene Derivatives

A mixture of arylaldehyde (1 mmol), active methylene compound (dimedone) (2 mmol), and Amberlite catalyst (0.4 mmol) in acetonitrile (10 ml) were refluxed until the limiting reagent was completed. The disappearance of the starting materials was ascertained by TLC by using *n*-hexane–ethyl acetate (1:1) solvent system. After completion of the reaction, the mixture were allowed to cool to room temperature and was then filtered. Then the reaction mixture was dried over $MgSO_4$ (anhydrous) and vaporized under vacuum to remove the solvent. The residue was also recrystallized by hot ethanol and was then filtered and washed with ethanol to afford the pure fraction in excellent yield. Then the crystalline fraction was further purified with column chromatography was done for the dried crystal two times. The fraction was applied on column chromatography and eluted with *n*-hexane/ethyl acetate (1:2) ratio to remove the arylaldehyde. The column was then eluted with *n*-hexane/ethyl acetate (1:1) ratio to remove the active methylene compound. Then after the solution was vaporized under vacuum and then the residue

was washed with hot ethanol to remove the excess reagents and then cooled to room temperature and was filtered by washing with cold ethanol and the residue were collected and dried. The same reaction was repeated by changing Amberlite catalysts *viz.* Amberlite IRA 67 and Amberlite IRC 86 (Nisar *et al.*, 2013). The products compounds (**3a-3c**) mass were measured and fully characterize by IR, ^{1}H NMR, ^{13}C NMR and DEPT-135.



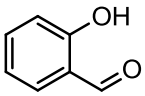
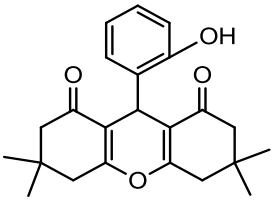
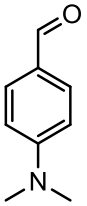
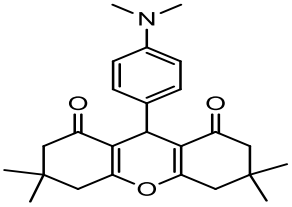
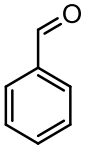
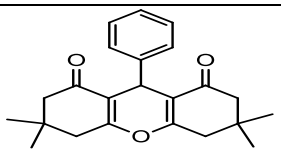
Entry	**Ar - CHO**	**Title Compounds**
1		 **3a**
2		 **3b**
3		 **3c**

Figure 15. General chemical reaction for the Synthesis of 1,8-Dioxooctahydroxanthene derivatives **(3a-3c)**

3.6. Test for Homogeneity (Purity) of the Compounds

The purity of each of the synthesized compounds were ascertained by analyzing with thin layer chromatography on silica gel thin layers with single and two-component developing solvents. The solvents were *n*-hexane and ethyl acetate taken in 1:1 and 1:2 ratios, respectively. The impure products were purified using column chromatography. Developing solvents used for column chromatography were the same as TLC (*n*-hexane- ethyl acetate) and also in 1:1 and 1:2 v/v ratios in respective order.

3.6.1. Characterization of Products

The following methods were employed for characterization and elucidation of structures of the new products of 1,8-Dioxo-octahydroxanthane derivatives.

- ✓ The melting points were determined in open glass capillaries.
- ✓ Infrared (IR) spectroscopy: Infrared spectra of each sample was recorded in 500 cm^{-1} to 4000 cm^{-1} range in KBr medium to identify and verify the presence of different functional groups and bonds in the products.
- ✓ Nuclear magnetic resonance (NMR) spectroscopy: Both ^{13}C and ^{1}H NMR were recorded to confirm the structure of the synthesized products.

3.7. Antimicrobial Assay

All the synthesized compounds were tested *in vitro* for their antibacterial activities against four bacterial strains (two Gram negative and two Gram positive) and was assessed by using the disc diffusion method. *In vitro* antimicrobial activity was screened using MHA. *Escherichia coli, Shigella flexineri, Streptococcus agalactia and Staphylococcus aureus* bacteria and antifungal activity against two fungal species, *Aspergillus niger* and *Fusarium oxysporum* fungi by paper disc diffusion method (Amare Ayelew, 2002). In bactericidal and fungicidal studies, Mueller hinton agar (MHA) and Potato dextrose agar (PDA) media, respectively were used. Known antibiotic Chloramphenicol was used as standard drug as reference in bactericidal and Bavistin was used as a standard drug against fungicidal assay. From inhibition zone data antimicrobial effects were estimated .

3.7.1. Preparation of Inoculums

The test bacterial strains, two gram-positive bacteria *Staphylococcus aureus and Streptococcus agalactia (S. aureus and Strepto. agalactia*) and two gram-negative bacteria *Escherichia coli and Shigella (E. coli and Shigella)* were transferred from the stock cultures and streaked on Muller hinton plates and incubated for 24 h at 37 ^{o}C. Well separated bacterial colonies were then used as inoculums. Bacteria were transferred using bacteriological loop to autoclaved Muller hinton agar that was cooled to about 45 ^{o}C in a water bath and mixed by gently swirling the flasks. The medium was then poured to sterile Petri plates, allowed to solidify and used for the bio-test (Nwinyi *et al.,* 2009).

For test fungi, mycelia plugs from stock cultures were transferred to PDA plates and incubated for 3 days. Then spores of *Aspargillus niger* and *Fusarium oxysporum* fungi each were harvested by washing the surface of the colony using 10 ml sterile distilled water and transferred to 250 ml autoclaved PDA cooled to about 45 °C in a water bath differently. The medium containing spore or mycelia suspension was poured to sterile plates, allowed to solidify and was used for the disc diffusion bioassay. Known antibiotics such as *Chloramphinicol* were used as standard drug as reference in bactericidal studies and in fungicidal studies Bavistin were used. From inhibition zone data correlations of structures with antimicrobial activity of compounds was critically examined. Finally antimicrobial activity of each compound were examined by measuring diameters of Inhibition zones (Nwinyi *et al.,* 2009).

3.7.2. Preparation of Sample Solutions

Standard solutions of all the samples were prepared by dissolving 10 mg of the sample products in 1 ml of *n*-hexane-ethylacetate(1:1) ratio and used for testing.

3.7.3. Testing for Antifungal Activity

Paper discs of about 6 mm in diameter were cut from whatman-No.1 filter paper with an office paper punch and placed in a beaker and covered with aluminum foil and sterilized in an oven at 180 ^{o}C for 1h. Then 20 µl of solutions of compounds were pipetted to the discs in three replications. After allowing the solvent to evaporate, the paper discs impregnated with the sample solutions were then transferred with sterile forceps to Potato Dextrose Agar seeded with

spore suspension of test fungi as described above. The Petridishes were incubated at 25 ^{o}C for 3 days. The entire test was performed in triplicate. The antifungal activity was evaluated by measuring the zone of inhibition against the test organism and solvent as positive control.

3.7.4. Testing for Antibacterial Activity

Similar procedures were followed that were done for antifungal activities. But in antibacterial activities instead of Potato Dextrose Agar the paper discs were transferred to Mueller Hinton Agar plate seeded with bacteria and incubated at 37 ^{o}C for 24h.

4. RESULTS AND DISCUSION

4.1. Synthesis of the Title Compound in Both Catalysts

The synthesis of the title compounds by using Amberlite IRA 67 and Amberlite IRC 86 catalysts, their reaction times percentage of yield and melting points are summarized in the tables 3 & 4.

Table 3. Amberlite IRA 67 Catalyzed Synthesis of 1, 8-Dioxooctahydroxanthene derivatives **(3a-3c)**

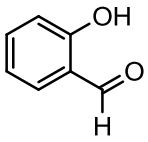
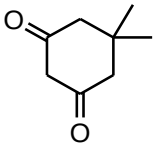
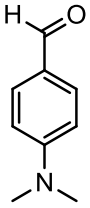
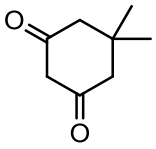
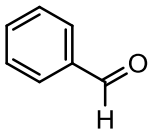
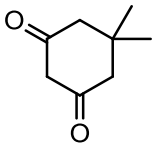
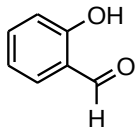
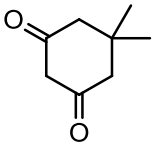
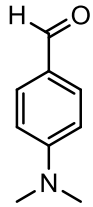
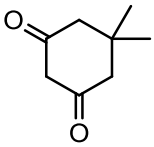
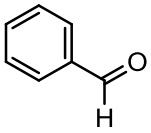
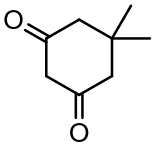
Entry	Ar-CHO	AMC	Product	Time (min.)	Yield (%)	M.P. (^{o}C)	
						Observed	Reported
1			**3a**	65	92	201-203	201-204 (Amit *et.al.* 2011), 203-205 (Kumar *et al.*, 2006)
2			**3b**	60	94	198-200	182-184 (Amit *et.al.* 2011), 221-224 (Shahram, *2014*)
3			**3c**	70	87	194-197	196-198 (Amit *et.al.* 2011), 203-205 (Shahram, *2014*)

Table 4. Amberlite IRC 86 Catalyzed Synthesis of 1, 8-Dioxooctahydroxanthene derivatives **(3a-3c)**

Entry	Ar-CHO	AMC	Product	Time (min.)	Yield (%)	M.P. (^{o}C)	
						Observed	Reported
1			**3a**	105	89	202-205	201-204 (Amit *et.al.* 2011), 203-205 (Kumar *et al.*, 2006)
2			**3b**	90	90	195-198	182-184 (Amit *et.al.* 2011), 221-224 (Shahram, *2014*)
3			**3c**	120	85	196-199	196-198 (Amit *et.al.* 2011), 203-205 (Shahram, *2014*)

4.2. Structural Elucidation of the Synthesized Compounds

3,4,6,7-tetrahydro-9-(2-hydroxyphenyl)-3,3,6,6-tetramethyl-2H-xanthene-1,8(5H,9H)-dione. (3a) white solid (89% yield), M.P. 202-205 ^{o}C. IR (KBr) (Vmax/ cm^{-1}): 3192 (O-H str), 2953.5 (C-H str), 1716 (C=O str), 1643 (C=C str), 1544 (C=C str), 1232 (C-O str), 1024 (C-C str).

Table 5. ^{1}H NMR Chemical shift values (δ) of 3,4,6,7-tetrahydro-9-(2-hydroxyphenyl)-3,3,6,6 tetramethyl-2H-xanthene-1,8(5H,9H)-dione in **(3a)** $CDCl_3$.

^{1}H –NMR δ(ppm)	No of hydrogen, multiplicity	Remark
1.01	6H, S	H-19 & H-20
1.036	3H, s	H-17
1.14	3H, s	H-18
1.99	2H, s	H-10
1.96	2H, s	H-6
2.39	2H, s	H-12
2.35	2H, s	H-4
2.52	1H, s	H-1
4.69	S	Hydroxyl proton
7.01	1H, s	H-21
7.03	1H, s	H-23
7.02	1H, s	H-25
7.04	1H, s	H-24

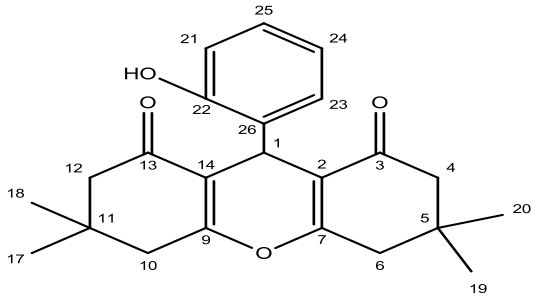
Table 6. ^{13}C NMR Chemical shift values (δ) of 3,4,6,7-tetrahydro-9-(2-hydroxyphenyl)-3,3,6,6-tetramethyl-2H-xanthene-1,8(5H,9H)-dione in **(3a)** $CDCl_3$.

^{13}C NMR δ(ppm)	Carbon Number	Remark
27.05	17,18	CH_3
27.72	19,20	CH_3
29.27	1	CH
31.0	5	Quaternary
32.3	11	Quaternary
41.5	6	CH_2
43.2	10	CH_2
50	4	CH_2
54	12	CH_2
101	2	Quaternary
111	14	Quaternary
115.8	21	CH
118.3	24	CH
124.3	26	Quaternary
124.6	25	CH
127.5	23	CH
128	22	Quaternary
169.2	7	Quaternary
171	9	Quaternary
196.9	3	Quaternary
200.1	13	Quaternary

Cyclocondensation of aryl aldehyde with dimedone leads to the formation of 3,4,6,7-tetrahydro-9-(2-hydroxyphenyl)-3,3,6,6-tetramethyl-2H-xanthene-1,8(5H,9H)-dione **(3a)** which is 1, 8-dioxo-octahydroxanthene derivative containing C=O,C=C, C-C, O-H, C-O, CH_2 and CH groups of heterocyclic ring with the elimination of a water molecule. The main IR spectra of these compounds exhibit υ O-H, υ C-H, υ C=O, υ C=C, υ C-O and υ C-C vibrations in 3192 cm^{-1}, 2953.5 cm^{-1}, 1716 cm^{-1}, 1643 & 1544 cm^{-1}, 1375 cm^{-1}, 1232 cm^{-1} and 1024 cm^{-1} regions, respectively (Appendix Figure 2).

Additionally infrared inferences regarding the structures of the synthesized compounds, ^{1}H, ^{13}C and DEPT-135 NMR spectra have been critically examined. ^{1}H NMR spectra (Appendix figure 3) displayed signals for all common proton containing groups, CH_3 (chain), CH_2 (ring), CH (ring) and benzene ring, in δ value 1.01-1.14, 1.99-2.35, 2.52 and 7.01-7.04 ppm, respectively. In addition to this the hydroxyl group (-OH) hydrogen displayed the signal at 4.69 ppm. From the ^{13}C NMR (shown table 6) and DEPT-135 spectra as shown (Appendix Figure 4), the compound have ten quaternary carbon atoms at δ 31.0, 32.3, 101.0, 111.0, 124.3, 128.0, 169.2, 171.0, 196.9 and 200.1; and the DEPT-135 NMR also displayed downward peaks at δ 41.5, 43.2, 50 and 54.

In the DEPT-135 spectra as shown appendix figure 4 although all the signals for CH_2 (ring) are occurring at the same ppm as observed in ^{13}C NMR spectra but instead of downward position as expected some of the signals appeared in upward position; this upward appearance of signals of these groups might be due to enolization of C=O group by shifting of one hydrogen from adjacent methylene group (CH_2) in the ring. The number and chemical shift values of signals of CH (ring) and benzene rings are identical to ^{13}C NMR spectra.



3a

Figure 16. Structure of 3,4,6,7-tetrahydro-9-(2-hydroxyphenyl)-3,3,6,6-tetramethyl-2H-xanthene-1,8(5H,9H)-dione **(3a)**

9-(4-(dimethylamino)phenyl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethyl-2H-xanthene-1,8(5H,9H)-dione. (3b) yellowish gray (90% yield), M.P. 195-198 ^{o}C. IR (KBr) (*V*max/ cm^{-1}): 2960 (C-H str), 1739 (C=O str), 1587 (C=C str), 1371 (C-N str), 1158 (C-O str), 811 (C-C str).

Table 7. ^{13}C NMR Chemical shift values (δ) of 9-(4-(dimethylamino)phenyl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethyl-2H-xanthene-1,8(5H,9H)-dione **(3b)** in $CDCl_3$.

^{13}C -NMR δ(ppm)	Carbon Number	Remark
31.39	15, 16, 19, 20	CH_3
31.849	2, 12	Quaternary
40.71	10	CH
46.48	28, 29	CH_3
47.09	3, 11	CH_2
60.39	1, 13	CH_2
111.02	5, 9	Quaternary
112.67	21, 25	CH
115.96	22, 24	CH
125.61	23	Quaternary
127.50	26	Quaternary
148.76	4, 8	Quaternary
189.75	6, 14	Quaternary

Table 8. ^{1}H NMR Chemical shift values (δ) of 9-(4-(dimethylamino)phenyl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethyl-2H-xanthene-1,8(5H,9H)-dione **(3b)** in $CDCl_3$.

1H –NMR δ(ppm)	No of hydrogen, multiplicity	Remark
1. 12-1.25	12H, s	H-15, H-16, H-19, H-20
2.07	4H, s	H-3, H-11
2.92	6H, s	H-28, H-29
3.10	4H, s	H-1, H-13
4.13	1H, s	H-10
6.70	2H, d, J=8.8	H-21, H-25
6.90	2H, d, J=8.45	H-22, H-24

Cyclocondensation of aryl aldehyde with dimedone leads to the formation of 1, 8-dioxo-octahydroxanthene derivatives **(3b)** containing C=O, C=C, C-C,C-O, CH_2 and CH groups of heterocyclic ring with the elimination of a water molecule. The main IR spectra of these compounds exhibit υ C-H, υ C=O, υ C=C, υ C-N, υ C-O and υ C-C vibrations in 2960 cm^{-1}, 1739 cm^{-1}, 1587 cm^{-1}, 1371 cm^{-1}, 1158 cm^{-1} and 811 cm^{-1} regions, respectively (Appendix Figure1).

In order to verify infrared inferences regarding the structures of the synthesized compounds, ^{1}H, ^{13}C and DEPT-135 NMR spectra have been critically examined. ^{1}H NMR spectrum (Table 8) displayed signals for all common proton containing groups, CH_3 (chain), CH_2 (ring), CH (ring) and benzene ring, in δ value 1.12-1.25, 2.4-3.10, 4.13 and 6.7-6.97 ppm, respectively (Appendix Figure 8). In addition to this the CH_3(chain) group attached with N displayed the signal at 2.92 ppm. ^{13}C NMR (Table 7) and DEPT-135 spectra (Appendix Figure 6), showed the compound have ten quaternary carbon atoms at δ 125.6, 127.50, 31.85, 111.05, 148.76 and 189.75

(Appendix Figure 7). The DEPT-135 NMR also displayed downward peaks at δ 47.09 and 60.39 that indicates the CH_2 group (Appendix Figure 6)

In the DEPT spectrum as shown Appendix Figure 6 although all the signals for CH_2 (ring) are occurring at the same chemical shift region as observed in ^{13}C NMR spectra but instead of downward position as expected some of the signals appeared in upward position; this upward appearance of signals of these groups might be due to enolization of C=O group by shifting of one hydrogen from adjacent methylene group (CH_2) in the chain as well as in the ring. The number and chemical shift values of signals of CH (ring) and benzene rings are identical to ^{13}C NMR spectra.

The inferences regarding structures of the synthesized compounds obtained from infrared spectral studies are strongly supported by ^{1}H NMR, ^{13}C NMR and DEPT-135 spectral data along with all the spectral data inferences confirm the following proposed structure of 9-(4-(dimethylamino)phenyl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethyl-2H-xanthene-1,8(5H,9H)-dione.

(3b)

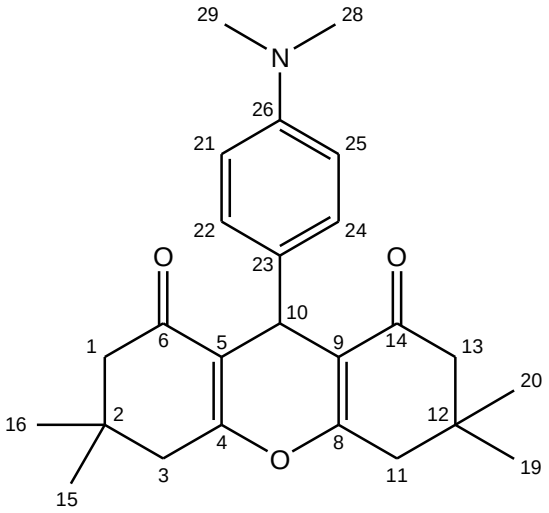


Figure 17. structure of 9-(4-(dimethylamino)phenyl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethyl-2H-xanthene-1,8(5H,9H)-dione. **(3b)**

4.3. Possible Reaction Mechanism of Synthesized Compounds

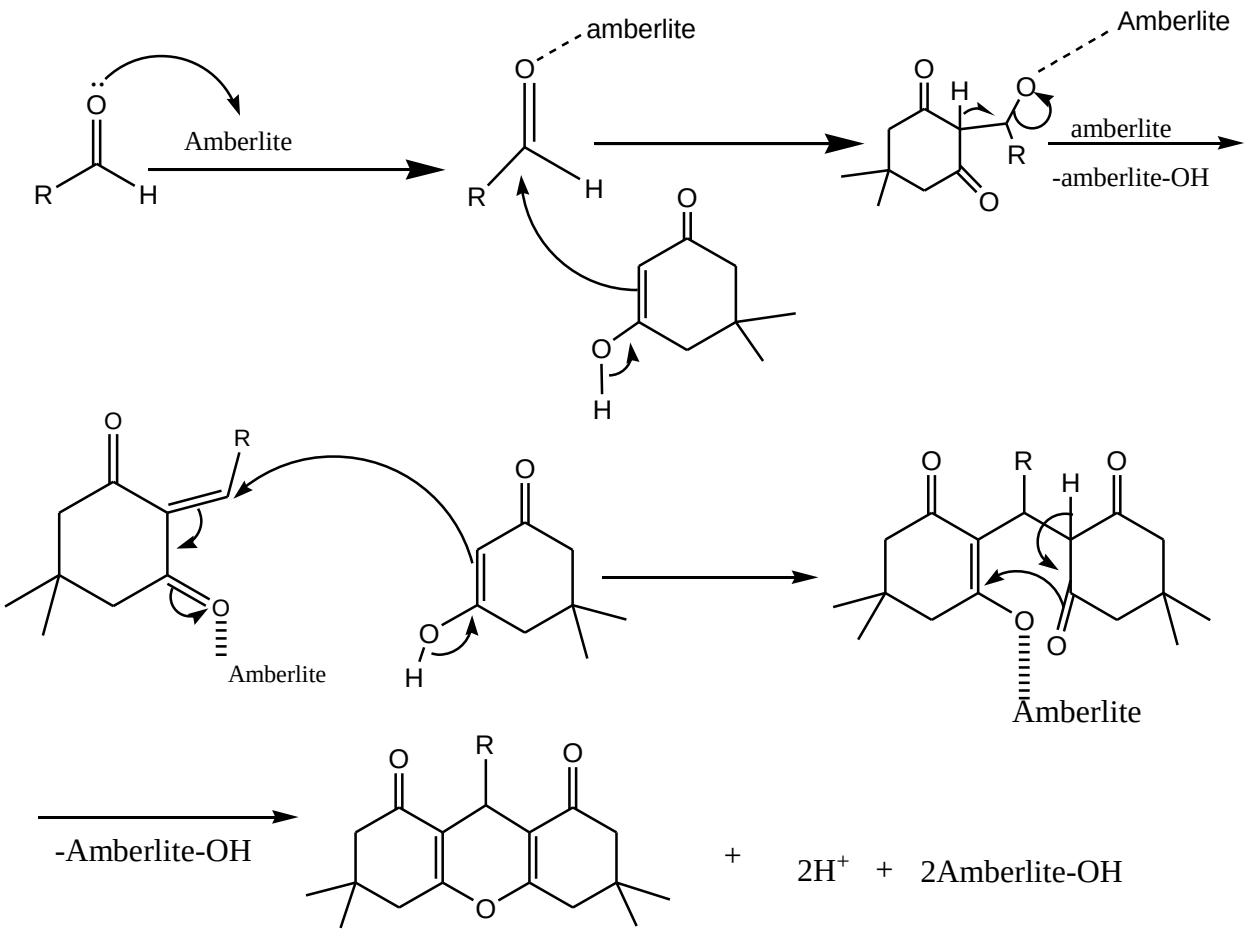


Figure 18. Reaction mechanism of 1, 8-dioxooctahydroxanthene derivatives **(3a-3c)**

4.4. Antimicrobial Activity of the Compounds

Table 9. The zone of inhibition (in mm) of 1, 8-dioxooctahydroxanthene derivatives: against tested bacteria strains by paper disc diffusion method in 20 µL/Disc.

Entry	Titled Compounds	Mean zone of inhibition (MZI) in mm			
		Gram negative bacteria		Gram positive bacteria	
		Shigella	*E. coli*	*S. aureus*	*S. agalactiae*
1	**3a**	21±0.78	22.7±0.7	22.5±0.9	-
2	**3b**	19.7±0.97	18.8±0.45	21.8±0.95	-
3	**3c**	15.3±0.2	15.7±0.3	17.2±0.85	-
4	Control	-	-	-	-
5	Standard control/ chloramphenicol	29.5±0.7	29.2±0.18	32±0.87	30±0.84

Key: '–' means no zone of inhibition

Table 10. The zone of inhibition (in mm) of 1, 8-dioxooctahydroxanthene derivatives: against tested fungi strains by paper disc diffusion method in 20 µL/Disc.

Entry	Titled Compounds	Mean zone of inhibition (MZI) in mm	
		Fusarium oxysporum	*Aspergillus niger*
1	**3a**	14.2±0.6	-
2	**3b**	12.8±0.45	-
3	**3c**	10.8±0.6	-
4	Control	-	-
5	Standard control/Bavistin	30.8±0.45	31±0.92

Key: '–' means no zone of inhibition

All the synthesized 1, 8-dioxo-octahydroxanthene derivatives have been tested for their antimicrobial (antibacterial and antifungal) activities against two Gram negative bacteria (*Escherichia coli* and *Shigella*) and two Gram positive bacteria (*Staphylococcus aureus* and *Streptococcus agalactiae*) (Table 9) and two fungi (*Fusarium oxysporum* and *Aspergillus niger*) (Table10). For this antimicrobial activity the commercial standard drugs Chloramphenicol and Tilt have been used as standard control/ reference for bacteria and fungicidal activities respectively.

The antibacteria activity of 1, 8-dioxooctahydroxanthene derivatives **(3a-3c)** show good inhibition for the two Gram negative bacteria (*Escherichia coli* and *Shigella*) and one Gram positive bacteria (*Staphylococcus aureus*); but for one Gram positive bacteria (*Streptococcus agalactiae*) didn't show any inhibition. On the other hand, the antifungal activity of 1, 8-dioxooctahydroxanthene derivatives show activity against *Fusarium oxysporum*, but didn't show any significant against Aspergillus *niger.*

Among all 1, 8-dioxooctahydroxanthene derivatives, two of them have better inhibition than any other 1, 8-dioxooctahydroxanthene derivatives against for all three bacteria and even the fungi. Those derivetives of the synthesized compounds are 9-(4-(dimethylamino)phenyl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethyl-2H-xanthene-1,8(5H,9H)-dione **(3b)** and 3,4,6,7-tetrahydro-9-(2-hydroxyphenyl)-3,3,6,6-tetramethyl-2H-xanthene-1,8(5H,9H)-dione **(3a)** which spectroscopic analysis have been done.

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5. 1. Summary and Conclusion

As a result of the present studies, derivatives of 1,8-dioxo-octahydroxanthene have been successfully synthesized by using Amberlite IRA-67 and IRC-86 as an environmentally friendly catalysts. Its use with acetonitrile increases its compatibility in organic synthesis. This method is applicable to the functionalization of the aromatic moiety of aldehydes with a wide range of substituents, and provides the corresponding 1,8-dioxooctahydroxanthene derivatives in good to excellent yields. The present protocol offers several advantages including reduced reaction times, the economic viability of the catalyst and a simple reaction work up. Remarkably, the required starting materials are readily available and relatively cheap, which is another advantage making this methodology extremely useful for the synthesis of such an important class of compounds.

For the "best set of conditions" comparison between both catalysts, we found that Amberlite IRA 67 has shown the best candidate catalyst characteristics with respect to percentage of the yield, reaction time, facile work up procedures and separation after the reaction compound.

All the synthesized compounds were evaluated for their antimicrobial activities such as antibacterial and antifungal, *in vitro* by using the paper disc diffusion technique. For antibacterial assay four microbes were used *viz. S. aures, E. coil, shigela and S. agalactiae* whereas antifungal studies were conducted against two fungi *viz. A. niger* and *F.oxysporum.*

5.2. Recommendations

With a view to contribute in the development of chemistry of heterocyclic organic and in the field of medicinal chemistry, the following recommendations are proposed**.**

- The antimicrobial studies can be extended to other bacteria and fungi except already tested microbes.
- The series of these compounds can be extended by synthesizing more compounds with different substitutents and with different starting materials and the synthesized compounds can be characterized by using aforesaid spectroscopic techniques.
- In medicinal chemistry, apart from these microbial studies on microbes, antimicrobial investigations can be extended on wide range of other microbes and their MIC (Minimum Inhibitory Concentrations) can be determined.
- To overcome the alarming problems of the world like HIV, tuberculosis and cancer, these novel active compounds can be tested for such hazardous diseases. Many other pharmacological properties like anticonvulsant, anti-inflammatory, anti-lishmenasis, anti-malaria etc. of the synthesized compounds can be studied.

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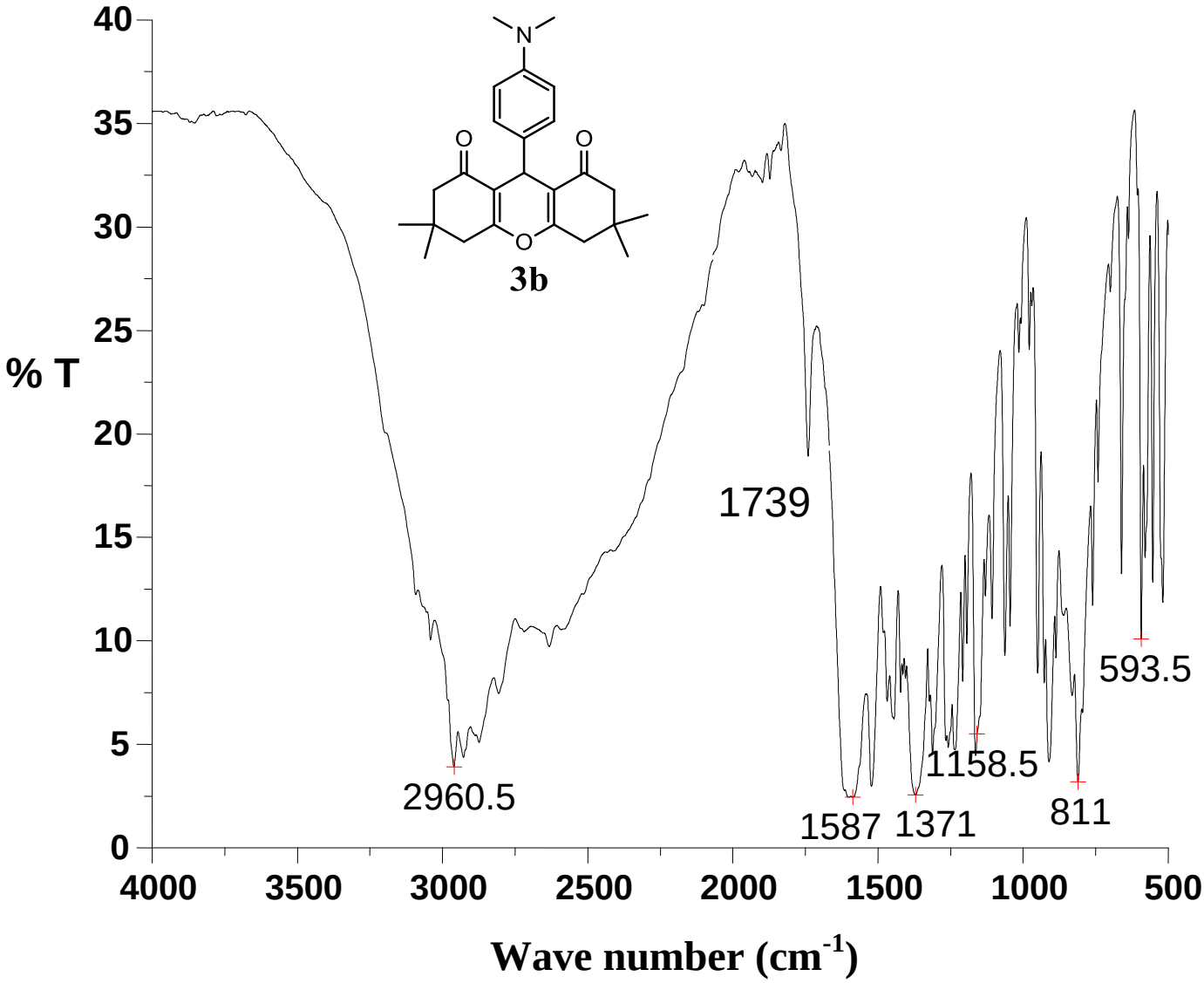
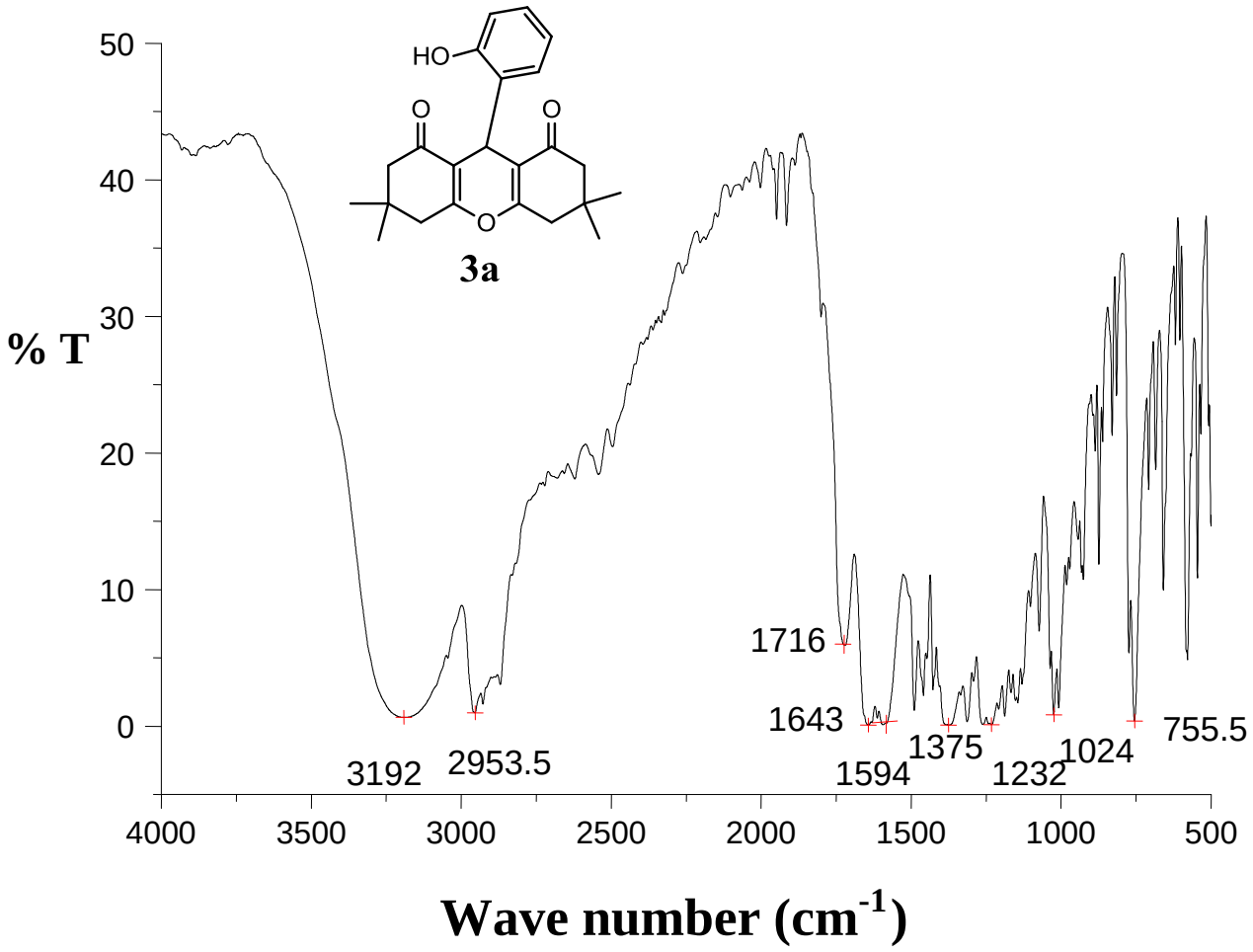
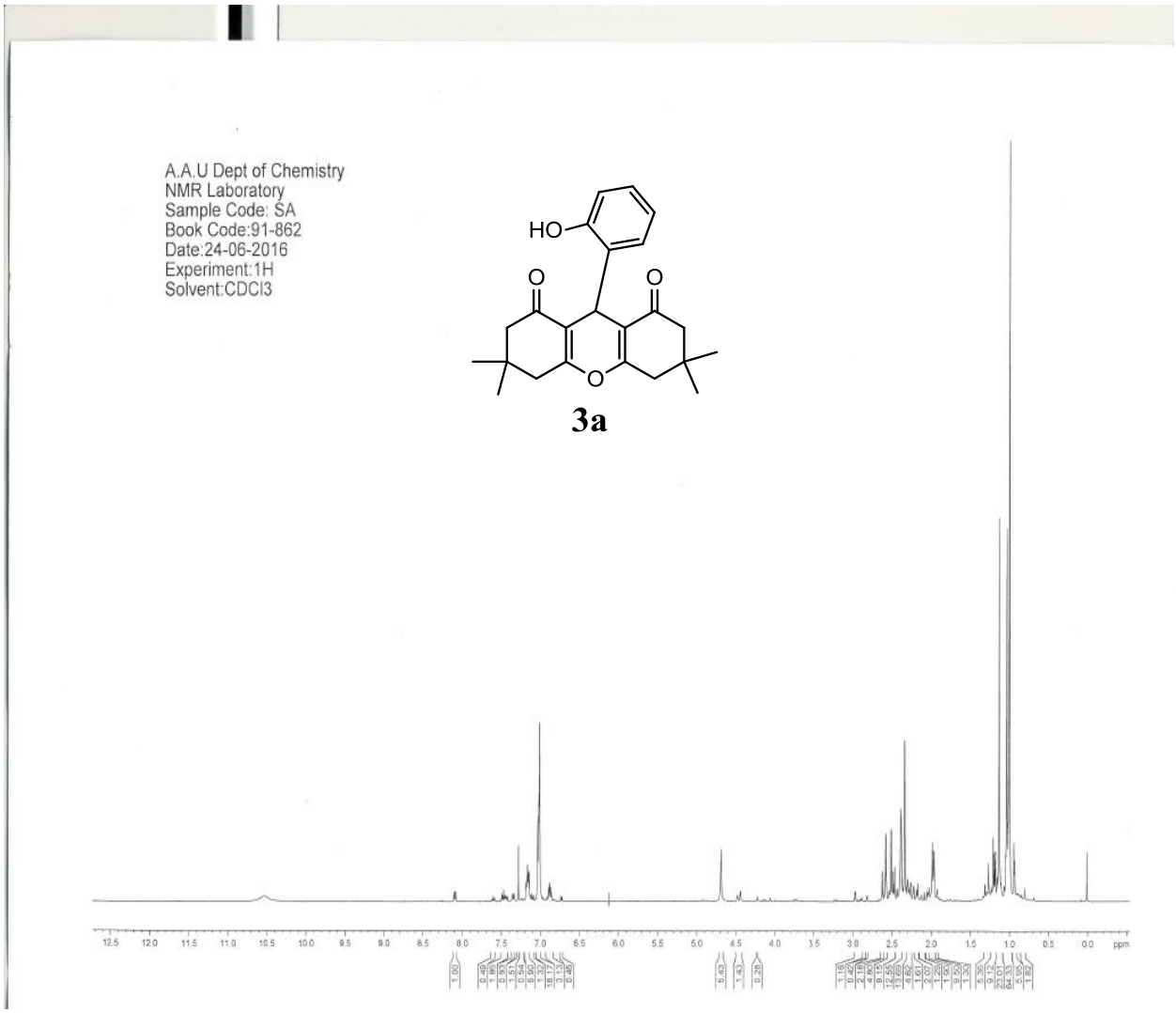
7. APENDIX



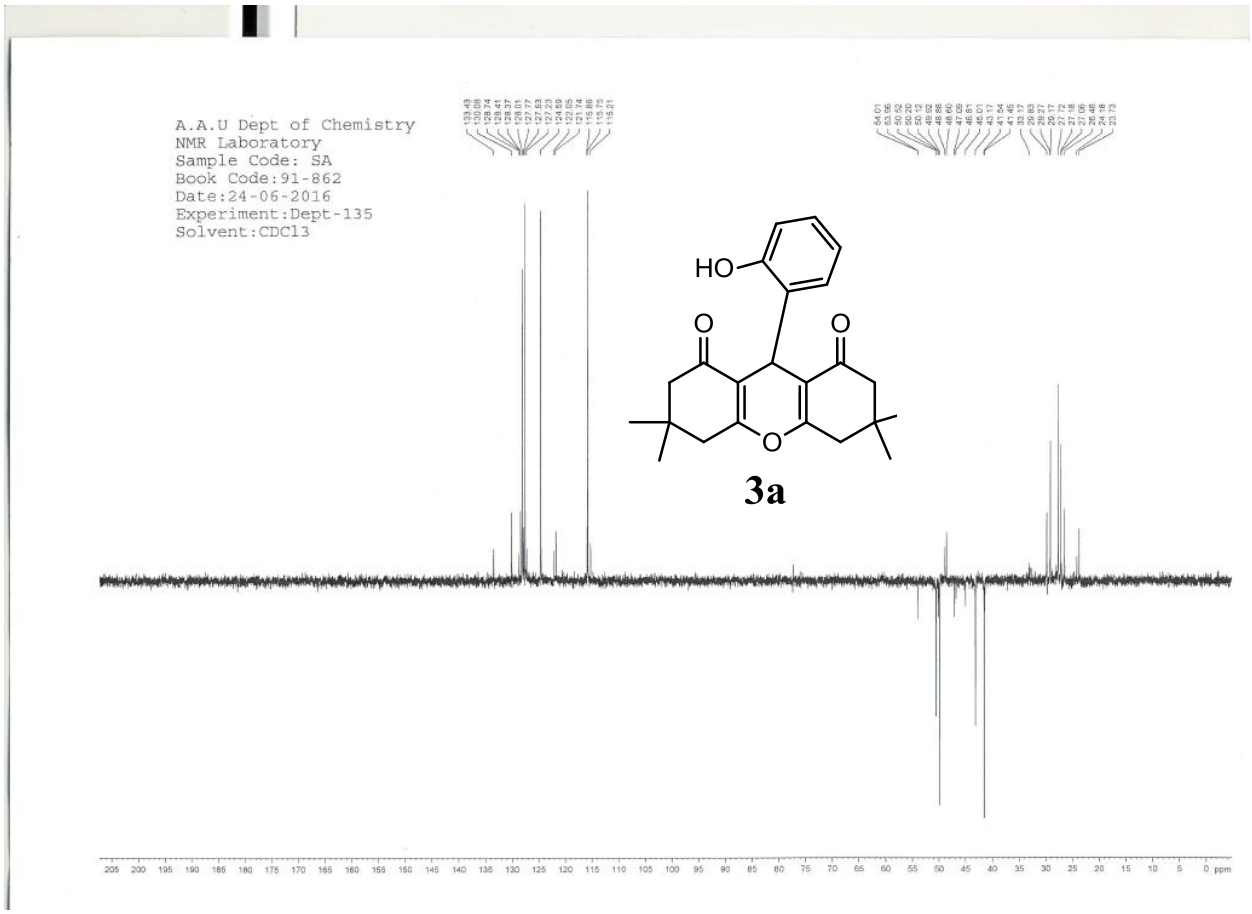
Appendix figure 1. FT-IR spectrum of 9-(4-(dimethylamino)phenyl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethyl-2H-xanthene-1,8(5H,9H)-dione **(3b)**



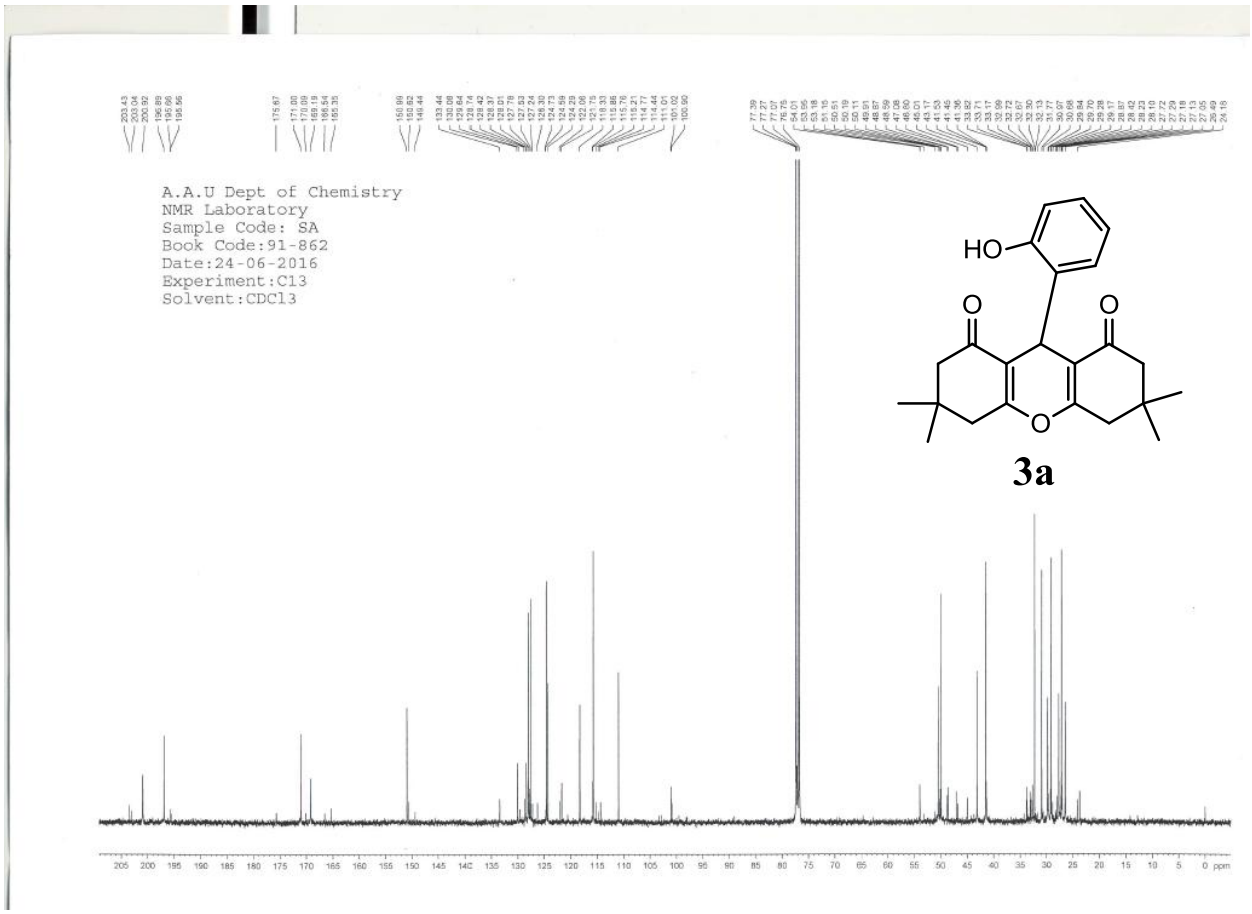
Appendix figure 2. FT-IR spectrum of 3,4,6,7-tetrahydro-9-(2-hydroxyphenyl)-3,3,6,6-tetramethyl-2H-xanthene-1,8(5H,9H)-dione **(3a)**



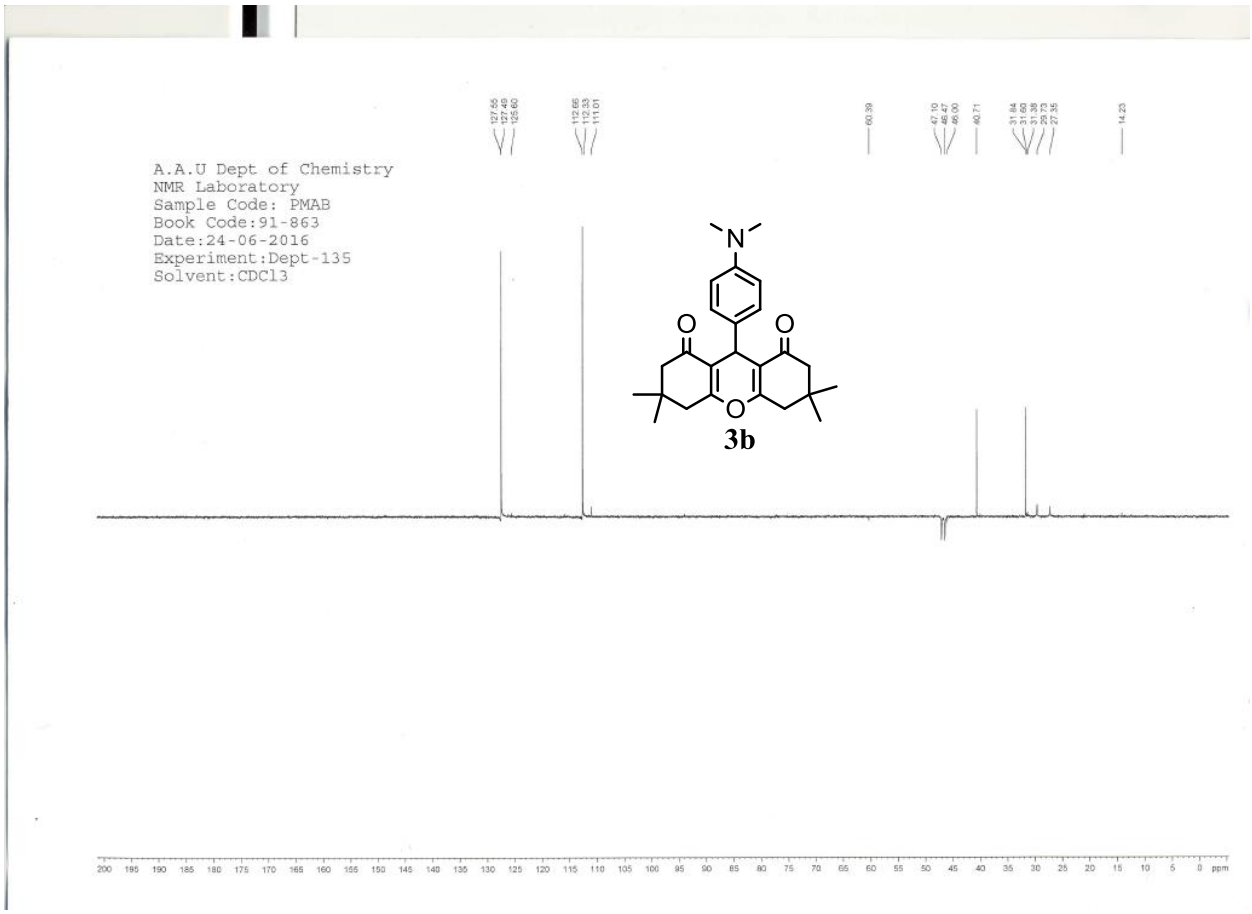
Appendix figure 3. ^{1}H NMR spectrum of 3,4,6,7-tetrahydro-9-(2-hydroxyphenyl)-3,3,6,6-tetramethyl-2H-xanthene-1,8(5H,9H)-dione in $CDCl_3$ **(3a)**



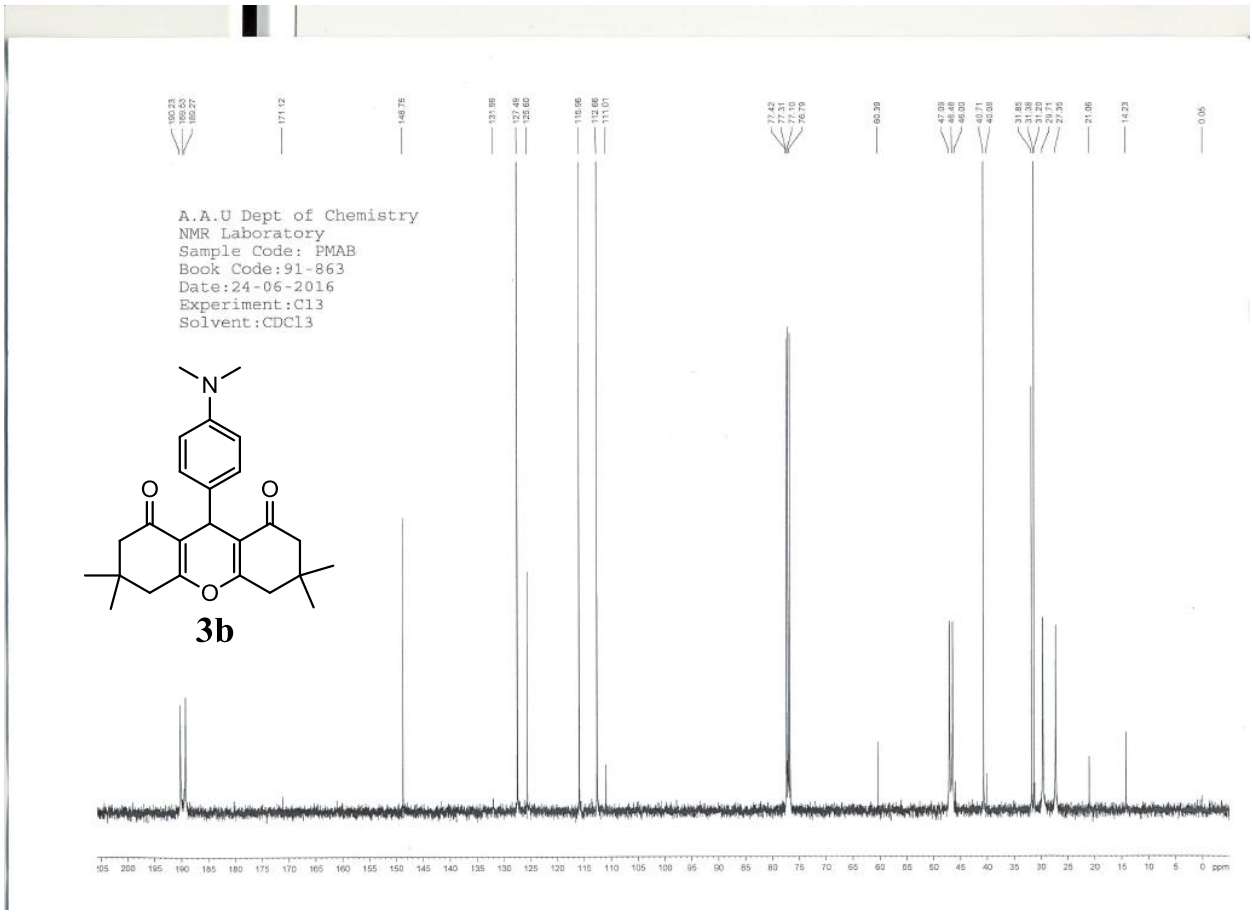
Appendix figure 4. DEPT-135 spectrum of 3,4,6,7-tetrahydro-9-(2-hydroxyphenyl)-3,3,6,6-tetramethyl-2H-xanthene-1,8(5H,9H)-dione in $CDCl_3$ **(3a)**



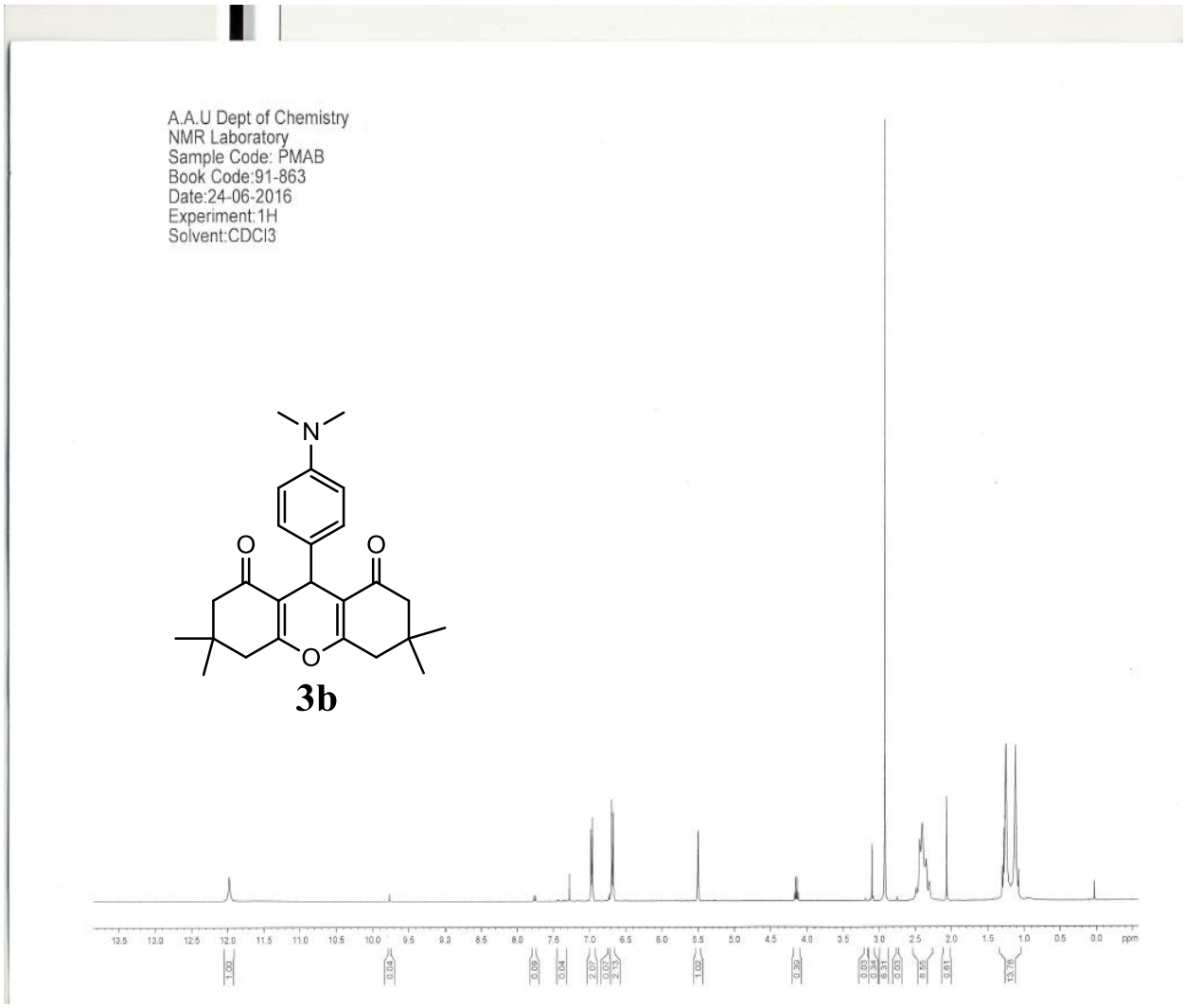
Appendix figure 5. ^{13}C NMR spectrum of 3,4,6,7-tetrahydro-9-(2-hydroxyphenyl)-3,3,6,6-tetramethyl-2H-xanthene-1,8(5H,9H)-dione in $CDCl_3$ **(3a)**



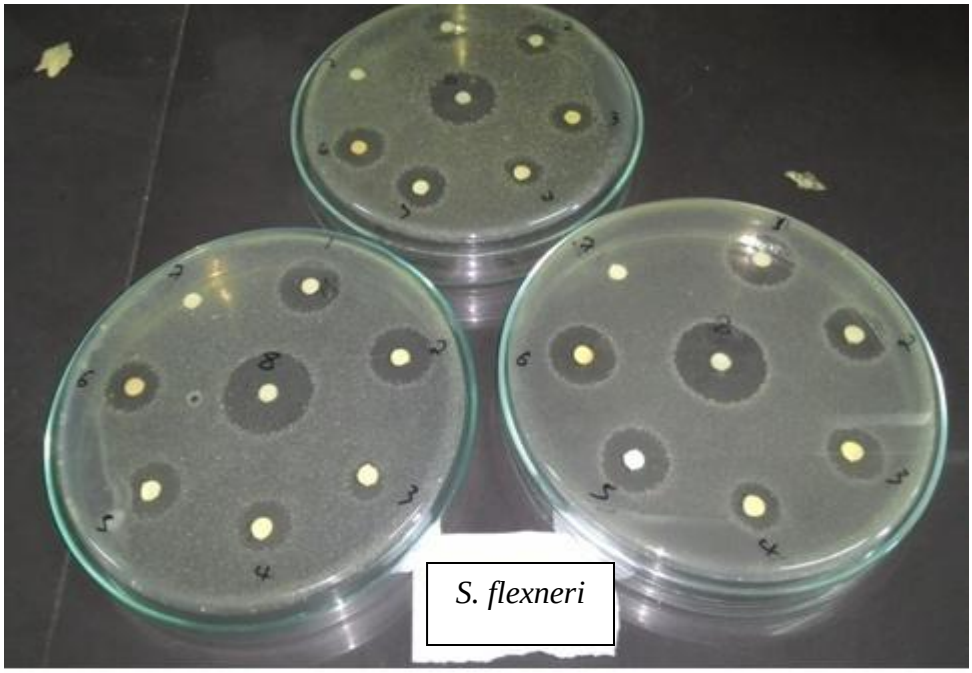
Appendix figure 6. DEPT-135 spectrum of 9-(4-(dimethylamino)phenyl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethyl-2H-xanthene-1,8(5H,9H)-dione in $CDCl_3$ **(3b)**



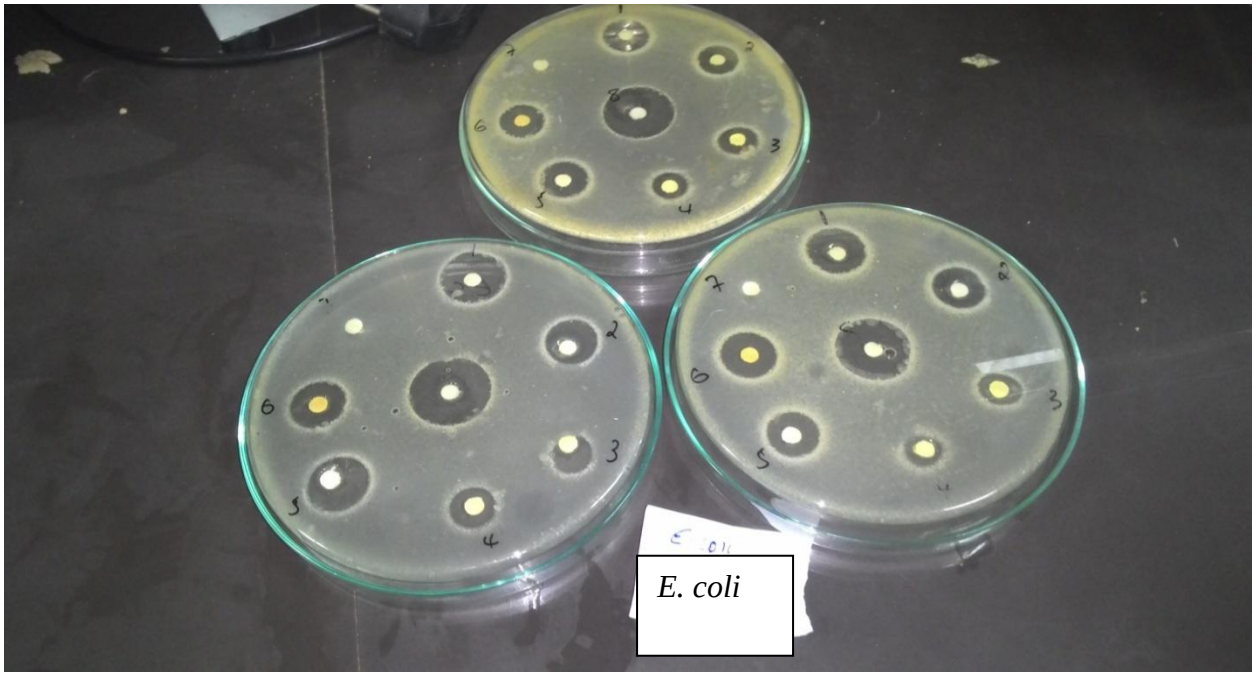
Appendix figure 7. ^{13}C NMR spectrum of 9-(4-(dimethylamino)phenyl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethyl-2H-xanthene-1,8(5H,9H)-dione in $CDCl_3$ **(3b)**



Appendix figure 8. ^{1}H NMR spectrum of 9-(4-(dimethylamino)phenyl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethyl-2H-xanthene-1,8(5H,9H)-dione in $CDCl_3$ **(3b)**

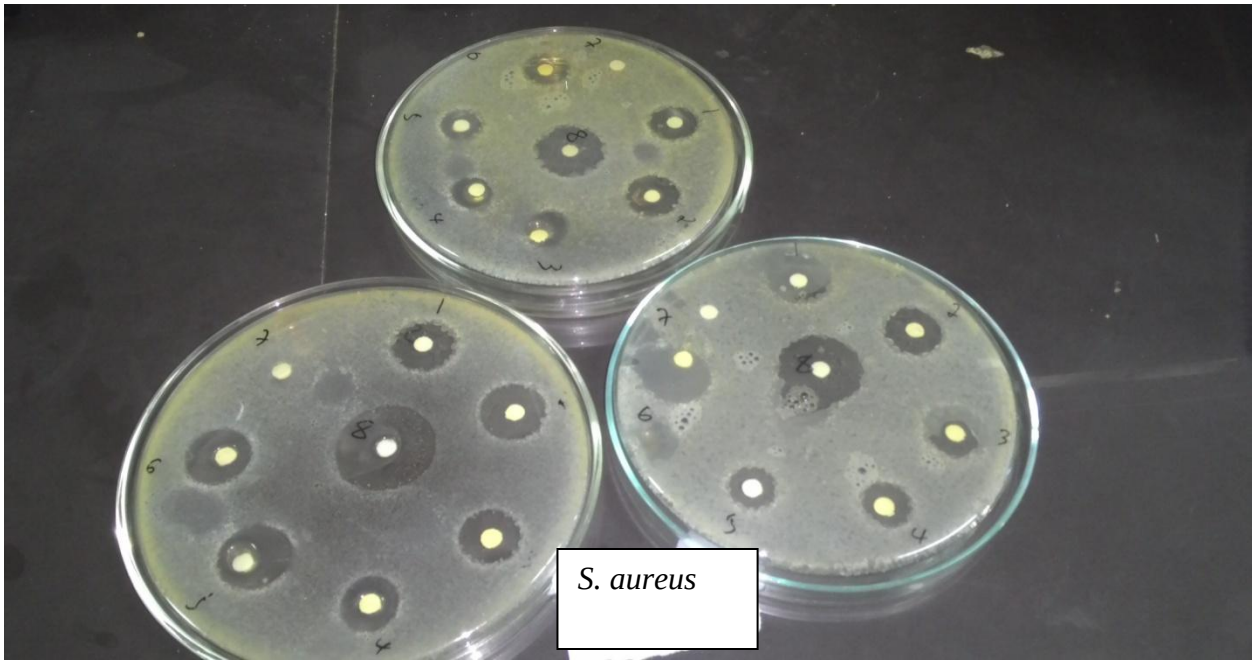


Shigella flexneri

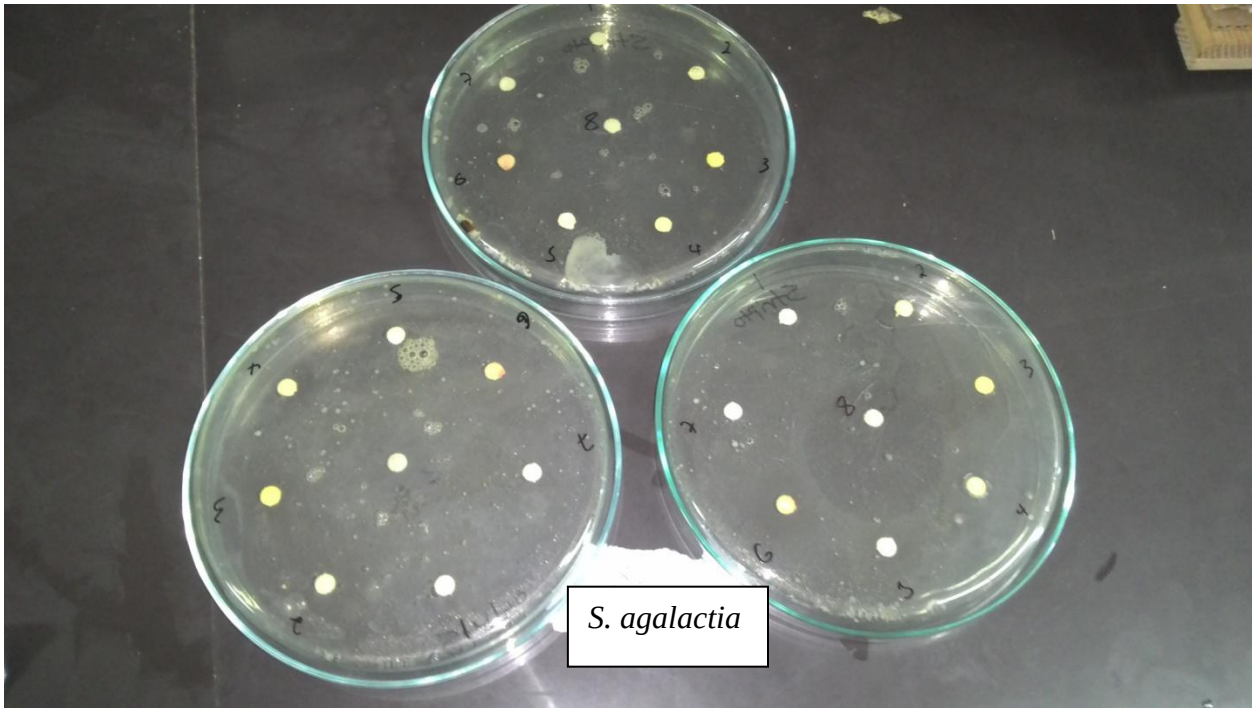


Escherichia coli

Appendix figure 9. Gram negative bacteria test of 1, 8 - Dioxooctahydroxanthene derivatives **(3a-3c)**

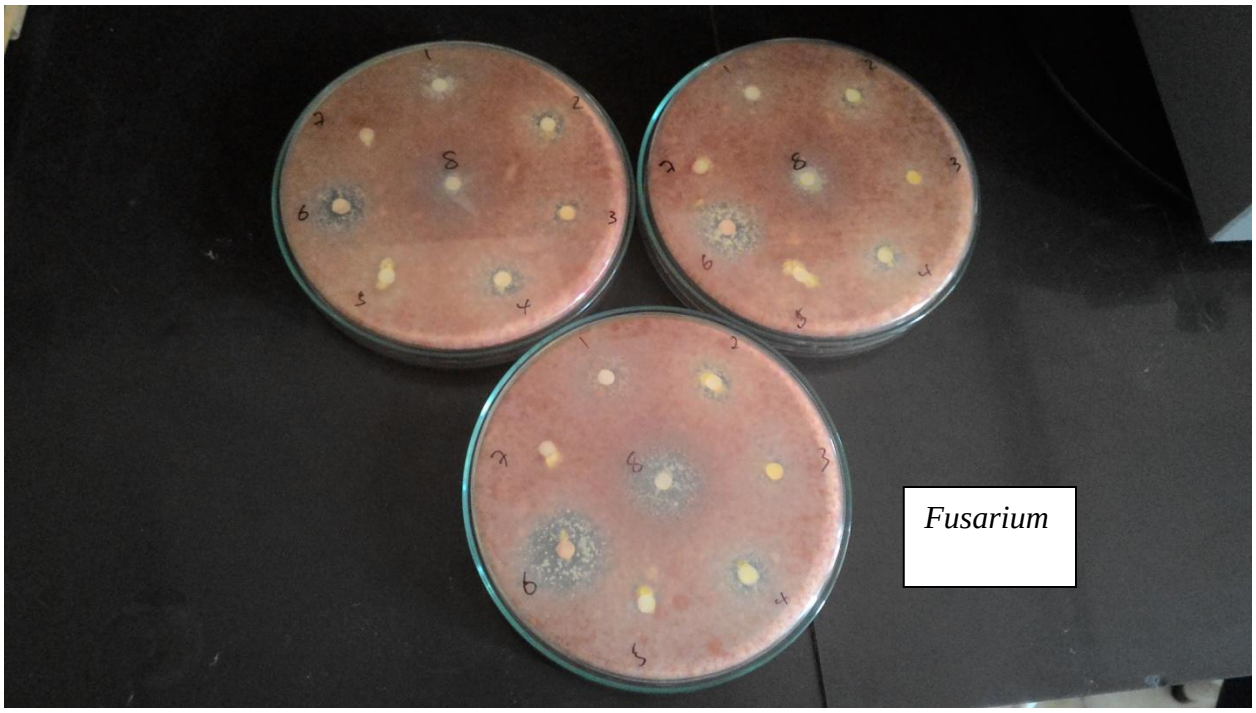


Staphylococcus aureus

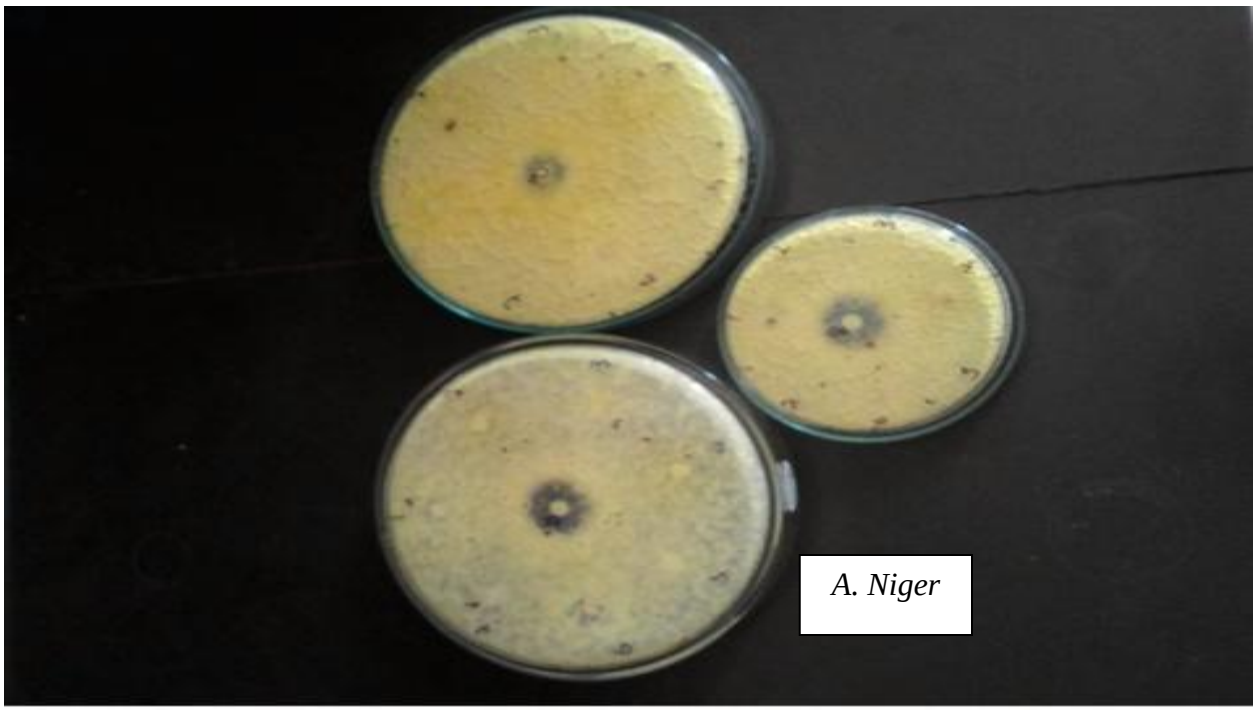


Streptococcus agalactiae

Appendix figure 10. Gram positive bacteria test of 1, 8 - Dioxooctahydroxanthene derivatives **(3a-3c)**



Fusarium oxysporum



Aspergillus Niger

Appendix figure 11. Anti fungi test of 1, 8-Dioxooctahydroxanthene derivatives **(3a-3c)**